



US011345903B2

(12) **United States Patent**
Mijts et al.

(10) **Patent No.: US 11,345,903 B2**
(45) **Date of Patent: May 31, 2022**

(54) **ENGINEERED ENZYMES**

(71) Applicant: **Inscripta, Inc.**, Boulder, CO (US)

(72) Inventors: **Benjamin Mijts**, Boulder, CO (US);
Juhan Kim, Boulder, CO (US); **Aamir Mir**,
Boulder, CO (US); **Kyle Seamon**,
Boulder, CO (US); **Andrew Garst**,
Boulder, CO (US)

(73) Assignee: **Inscripta, Inc.**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 76 days.

(21) Appl. No.: **16/953,253**

(22) Filed: **Nov. 19, 2020**

(65) **Prior Publication Data**

US 2021/0155912 A1 May 27, 2021

Related U.S. Application Data

(63) Continuation of application No. 16/844,079, filed on
Apr. 9, 2020, now Pat. No. 10,876,102, which is a
continuation of application No. 16/798,315, filed on
Feb. 22, 2020, now Pat. No. 10,640,754, which is a
continuation of application No. 16/658,948, filed on
Oct. 21, 2019, now Pat. No. 10,604,746.

(60) Provisional application No. 62/748,668, filed on Oct.
22, 2018.

(51) **Int. Cl.**
C12N 9/22 (2006.01)

(52) **U.S. Cl.**
CPC **C12N 9/22** (2013.01)

(58) **Field of Classification Search**
CPC **C12N 9/22**
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,391,582	B2	5/2002	Ying et al.
6,837,995	B1	1/2005	Vassarotti et al.
7,166,443	B2	1/2007	Walker et al.
8,332,160	B1	12/2012	Platt et al.
8,697,359	B1	4/2014	Zhang et al.
8,926,977	B2	1/2015	Miller et al.
9,260,505	B2	2/2016	Weir et al.
9,361,427	B2	6/2016	Hillson
9,499,855	B2	11/2016	Hyde et al.
9,776,138	B2	10/2017	Innings et al.
9,790,490	B2	10/2017	Zhang et al.
9,896,696	B2	2/2018	Begemann et al.
9,982,279	B1	5/2018	Gill et al.
9,988,624	B2	6/2018	Serber et al.
10,011,849	B1	7/2018	Gill et al.
10,017,760	B2	7/2018	Gill et al.
10,266,851	B2	4/2019	Chen
2002/0139741	A1	10/2002	Kopf
2004/0110253	A1	6/2004	Kappler et al.
2010/0076057	A1	3/2010	Sontheimer et al.
2014/0068797	A1	3/2014	Doudna et al.

2014/0199767	A1	7/2014	Barrangou et al.
2014/0273226	A1	9/2014	Wu et al.
2015/0098954	A1	4/2015	Hyde et al.
2015/0159174	A1	6/2015	Frendewey et al.
2015/0176013	A1	6/2015	Musunuru et al.
2015/0191719	A1	7/2015	Hudson et al.
2015/0225732	A1	8/2015	Williams et al.
2016/0024529	A1	1/2016	Carstens et al.
2016/0053272	A1	2/2016	Wurzel et al.
2016/0053304	A1	2/2016	Wurzel et al.
2016/0076093	A1	3/2016	Shendure et al.
2016/0102322	A1	4/2016	Ravinder et al.
2016/0168592	A1	6/2016	Church et al.
2016/0281053	A1	9/2016	Sorek et al.
2016/0289673	A1	10/2016	Huang et al.
2016/0298134	A1	10/2016	Chen et al.
2016/0354487	A1	12/2016	Zhang et al.
2017/0002339	A1	1/2017	Barrngou et al.
2017/0022499	A1	1/2017	Lu et al.
2017/0051310	A1	2/2017	Doudna et al.
2017/0073705	A1	3/2017	Chen et al.
2017/0191123	A1	7/2017	Kim et al.
2017/0211078	A1	7/2017	Kamineneni et al.
2017/0240922	A1	8/2017	Gill et al.
2017/0369870	A1	12/2017	Gill et al.
2018/0028567	A1	2/2018	Li et al.
2018/0052176	A1	2/2018	Holt et al.
2018/0073013	A1	3/2018	Lorenz et al.
2018/0112235	A1	4/2018	Li et al.
2018/0200342	A1	7/2018	Bikard et al.
2018/0230460	A1	8/2018	Gill et al.
2019/0017072	A1	1/2019	Ditommaso et al.
2019/0169605	A1	6/2019	Masquelier et al.

FOREIGN PATENT DOCUMENTS

EP	2395087	12/2011
EP	3199632	8/2017
WO	WO2002/010183	2/2002
WO	WO 2003/087341	10/2003
WO	WO 2010/079430	7/2010
WO	WO 2011/072246	6/2011

(Continued)

OTHER PUBLICATIONS

International Search Report and Written Opinion for International
Application No. PCT/US20/19379, dated Jul. 22, 2020, p. 1-10.
International Search Report and Written Opinion for International
Application No. PCT/US20/36064, dated Sep. 18, 2020, p. 1-16.
International Search Report and Written Opinion for International
Application No. PCT/US20/40389, dated Oct. 13, 2020, p. 1-12.
Arnak, et al., "Yeast Artificial Chromosomes", John Wiley & Sons,
Ltd., doi:10.1002/9780470015902.a0000379.pub3, pp. 1-10 (2012).
Woo, et al., "Dual roles of yeast Rad51 N-terminal domain in
repairing DNA double-strand breaks", *Nucleic Acids Research*,
doi:10.1093/nar/gkaa.587, vol. 48, No. 15, pp. 8474-8489 (2020).
Bao, et al., "Genome-scale engineering of *Saccharomyces cerevisiae*
with single-nucleotide precision", *Nature Biotechnology*, doi:10.
1038/nbt.4132, pp. 1-6 (May 7, 2018).
Dicarlo, et al., "Genome engineering in *Saccharomyces cerevisiae*
using CRISPR-Case systems", *Nucleic Acids Research*, 41(7):4336-
43 (2013).

(Continued)

Primary Examiner — Tekchand Saidha

(74) *Attorney, Agent, or Firm* — Sarah Brashears

(57) **ABSTRACT**

The present disclosure provides engineered RNA-guided
enzymes for editing live cells.

17 Claims, 17 Drawing Sheets

Specification includes a Sequence Listing.

(56)

References Cited

FOREIGN PATENT DOCUMENTS

WO	WO 2011/143124	11/2011
WO	WO 2013/142578	9/2013
WO	WO 2013/176772	11/2013
WO	WO 2014/018423	1/2014
WO	WO 2014/144495	9/2014
WO	WO 2016/110453	7/2016
WO	WO 2017/053902	3/2017
WO	WO 2017/078631	5/2017
WO	WO 2017/083722	5/2017
WO	WO 2017/106414	6/2017
WO	WO 2017/161371	9/2017
WO	WO 2017/174329	10/2017
WO	WO 2017/186718	11/2017
WO	WO 2017/216392	12/2017
WO	WO 2017/223330	12/2017
WO	WO 2018/031950	2/2018
WO	WO 2018/071672	4/2018
WO	WO 2018/083339	5/2018
WO	WO 2018/191715	10/2018
WO	WO2019/006436	1/2019

OTHER PUBLICATIONS

- Garst, et al., "Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering", *Nature Biotechnology*, 35(1):48-59 (2017).
- Hsu, et al., "DNA targeting specificity of RNA-guided Cas9 nucleases", *Nature Biotechnology*, 31(9):827-32 (2013).
- Jiang, et al., "RNA-guided editing of bacterial genomes using CRISPR-Cas systems", *Nature Biotechnology*, 31(3):233-41 (2013).
- Jinek, et al., "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity", *Science*, 337:816-20 (2012).
- Verwaal, et al., "CRISPR/Cpf1 enables fast and simple genome editing of *Saccharomyces cerevisiae*", *Yeast*, 35:201-11 (2018).
- Lian, et al., "Combinatorial metabolic engineering using an orthogonal tri-functional CRISPR system", *Nature Communications*, DOI: 1038/s41467-017-01695-x/www.nature.com/naturecommunications, pp. 1-9 (2017).
- Roy, et al., "Multiplexed precision genome editing with trackable genomic barcodes in yeast", *Nature Biotechnology*, doi:10.1038/nbt.4137, pp. 1-16 (2018).
- Dong, "Establishment of a highly efficient virus-inducible CRISPR/Cas9 system in insect cells", *Antiviral Res.*, 130:50-7(2016).
- Epinat et al., "A novel engineered meganuclease induces homologous recombination in eukaryotic cells, e.g., yeast and mammalian cells", *Nucleic Acids Research*, 31(11): 2952-2962.
- Farasat et al., "A Biophysical Model of CRISPR/Cas9 Activity for Rational Design of Genome Editing and Gene Regulation," *PLoS Comput Biol.*, 29:12(1):e1004724 (2016).
- Liu et al., "A chemical-inducible CRISPR-Cas9 system for rapid control of genome editing", *Nature Chemical Biology*, 12:980-987(2016).
- Eklund, et al., "Altered target site specificity variants of the I-PpoI His-Cys bis homing endonuclease" *Nucleic Acids Research*, 35(17):5839-50 (2007).
- Boles, et al., "Digital-to-biological converter for on-demand production of biologics", *Nature Biotechnology*, doi:10.1038/nbt.3859 (May 29, 2017).
- Pines, et al., "Codon Compression Algorithms for Saturation Mutagenesis", *ACS Synthetic Biology*, 4:604-14 (2015).
- Bessa et al., "Improved gap repair cloning in yeast: treatment of the gapped vector with Taq DNA polymerase avoids vector self-ligation," *Yeast*, 29(10):419-23 (2012).
- Boch, "TALEs of genome targeting," *Nature Biotechnology* vol. 29, pp. 135-136 (2011).
- Campbell et al., "Targeting protein function: the expanding toolkit for conditional disruption," *Biochem J.*, 473(17):2573-2589 (2016).
- Casini et al., "Bricks and blueprints: methods and standards for DNA assembly," *Nat Rev Mol Cell Biol.*, (9):568-76 (2015).
- Chica et al., "Semi-rational approaches to engineering enzyme activity: combining the benefits of directed evolution and rational design," *Current Opinion in Biotechnology*, 16(4): 378-384 (2005).
- Durai et al., "Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells", *Nucleic Acids Res.*, 33(18):5978-90 (2005).
- Kadonaga et al., "Regulation of RNA polymerase II transcription by sequence-specific DNA binding factors", *Cell*, 116(2):247-57 (2004).
- Lee et al., "Targeted chromosomal deletions in human cells using zinc finger nucleases", *Genome Res.*, 20(1): 81-9 (2009).
- Miller et al., "A Tale nuclease architecture for efficient genome editing", *Nature Biotechnology*, 29 (2): 143-8 (2011).
- Mittelman et al., "Zinc-finger directed double-strand breaks within CAG repeat tracts promote repeat instability in human cells", *PNAS USA*, 106 (24): 9607-12 (2009).
- Shivange, "Advances in generating functional diversity for directed protein evolution", *Current Opinion in Chemical Biology*, 13 (1): 19-25 (2009).
- Udo, "An Alternative Method to Facilitate cDNA Cloning for Expression Studies in Mammalian Cells by Introducing Positive Blue White Selection in Vaccinia Topoisomerase I-Mediated Recombination," *PLoS One*, 10(9):e0139349 (2015).
- Urnov et al., "Genome editing with engineered zinc finger nucleases", *Nature Reviews Genetics*, 11:636-646 (2010).
- International Search Report and Written Opinion for International Application No. PCT/US2018/053608, dated Dec. 13, 2018, p. 1-9.
- International Search Report and Written Opinion for International Application No. PCT/US2018/053670, dated Jan. 3, 2019, p. 1-13.
- International Search Report and Written Opinion for International Application No. PCT/US2018/053671, dated Sep. 26, 2018, p. 1-12.
- International Search Report and Written Opinion for International Application No. PCT/US2018/040519, dated Sep. 26, 2018, p. 1-8.
- International Search Report and Written Opinion for International Application No. PCT/US2019/026836, dated Jul. 2, 2019, p. 1-10.
- International Search Report and Written Opinion for International Application No. PCT/US2019/023342, dated Jun. 6, 2019, p. 1-34.
- International Search Report and Written Opinion for International Application No. PCT/US2019/030085, dated Jul. 23, 2019, p. 1-14.
- International Search Report and Written Opinion for International Application No. PCT/US20/24341, dated Jun. 19, 2020, p. 1-9.
- NonFinal Office Action for U.S. Appl. No. 16/399,988, dated Jul. 31, 2019, p. 1-20.
- First Office Action Interview Pilot Program Pre-Interview Communication for U.S. Appl. No. 16/024,831, dated Feb. 12, 2019, p. 1-37.
- NonFinal Office Action for U.S. Appl. No. 16/024,816 dated Sep. 4, 2018, p. 1-10.
- Final Office Action for U.S. Appl. No. 16/024,816 dated Nov. 26, 2018, p. 1-12.
- First Office Action Interview Pilot Program Pre-Interview Communication Preinterview for U.S. Appl. No. 16/454,865 dated Aug. 16, 2019, p. 1-36.
- Yoshioka, et al., "Development of a mono-promoter-driven CRISPR/Cas9 system in mammalian cells", *Scientific Reports*, Jul. 3, 2015, p. 1-8.
- Remaut, et al., "Plasmid vectors for high-efficiency expression controlled by the PL promoter of coliphage lambda," *Laboratory of Molecular Biology*, Apr. 15, 1981, p. 81-93.
- International Search Report and Written Opinion for International Application No. PCT/US2019/028821, dated Aug. 2, 2019, p. 1-14.
- International Search Report and Written Opinion for International Application No. PCT/US2019/028883, dated Aug. 16, 2019, p. 1-12.
- International Search Report and Written Opinion for International Application No. PCT/US2019/46526, dated Dec. 18, 2019, p. 1-17.
- International Search Report and Written Opinion for International Application No. PCT/US2018/34779, dated Nov. 26, 2018, p. 1-39.
- International Search Report and Written Opinion for International Application No. PCT/US19/57250, dated Feb. 25, 2020, p. 1-16.
- International Search Report and Written Opinion for International Application No. PCT/US19/47135, dated Jun. 11, 2020, p. 1-15.

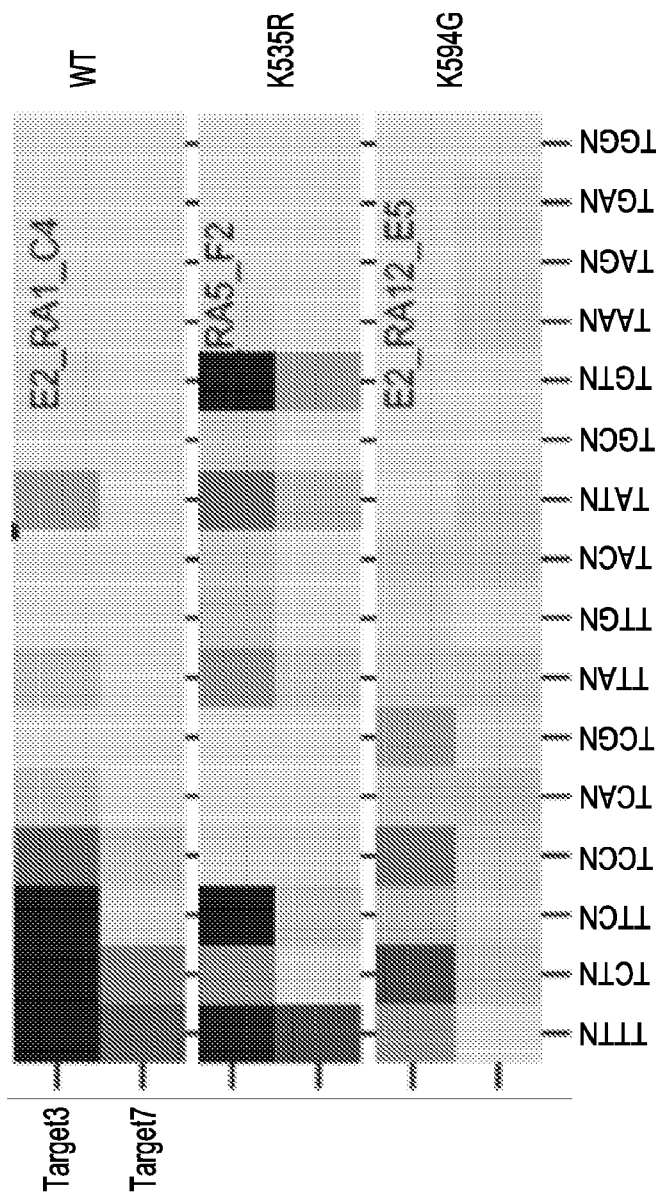


FIG. 1A

EXP50069-LIBRARY1A, HF-MAD7, SPECIFICITY PANEL

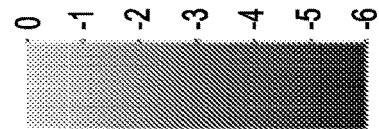
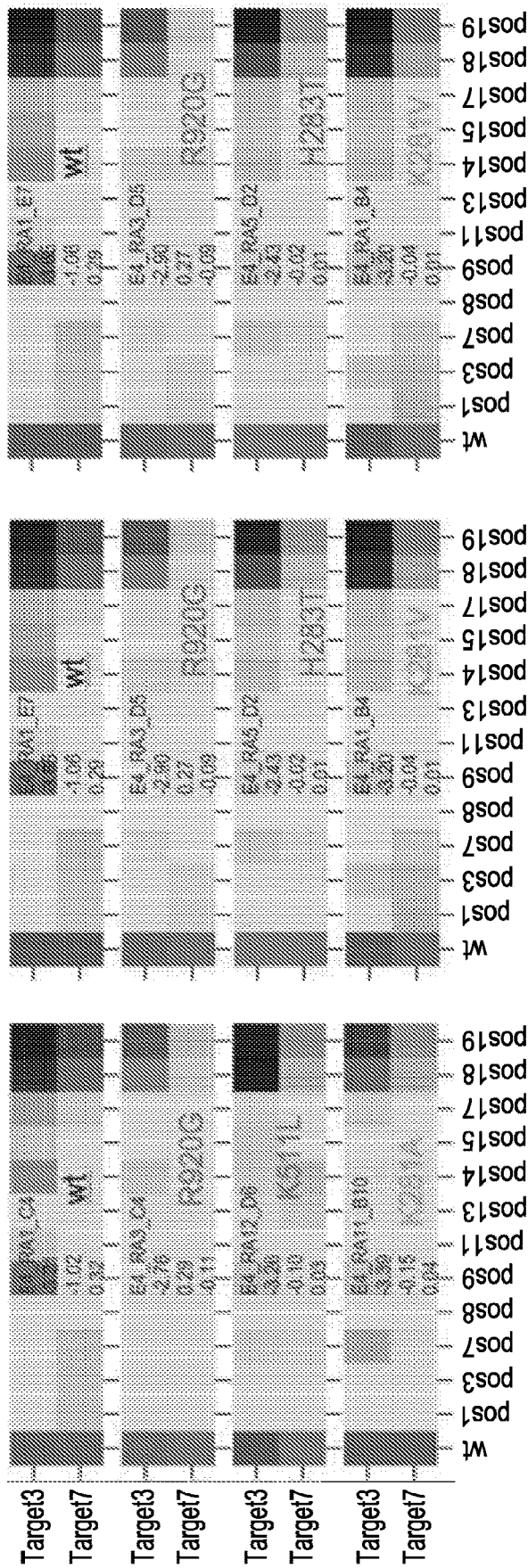


FIG. 1B-1

EXP50069-LIBRARY1A, HF-MAD7, SPECIFICITY PANEL

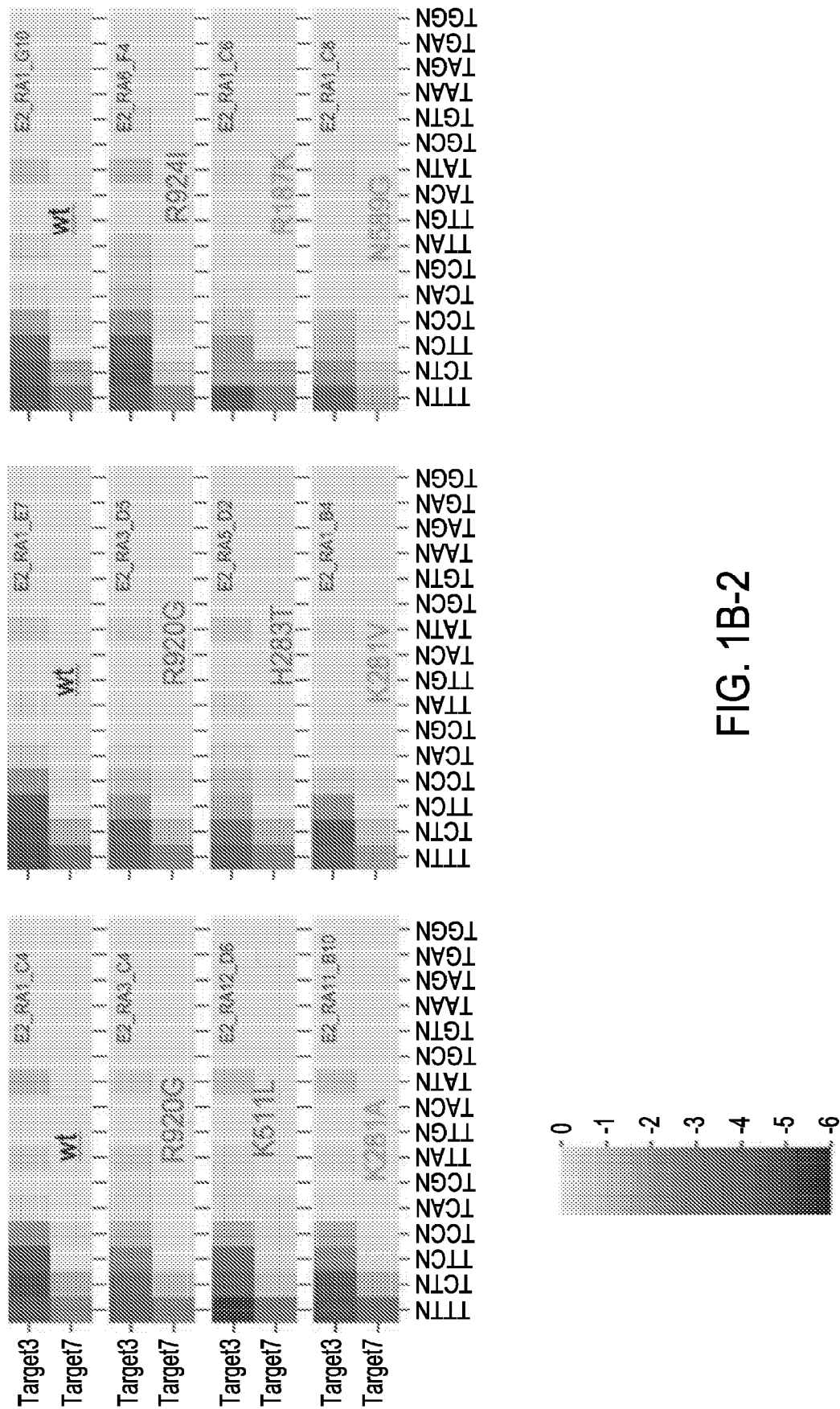


FIG. 1B-2

FIG. 2A

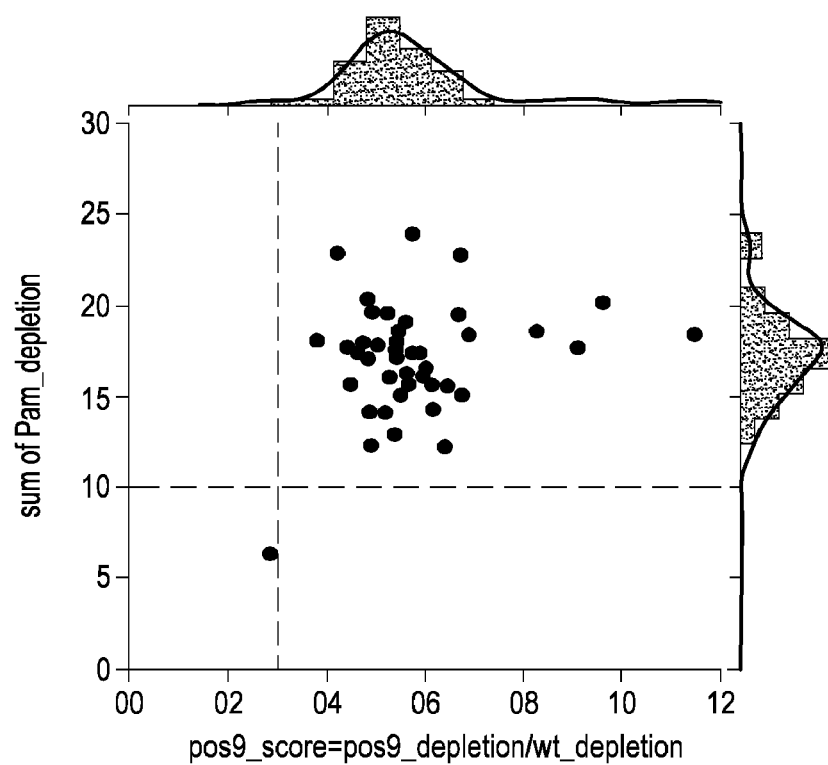
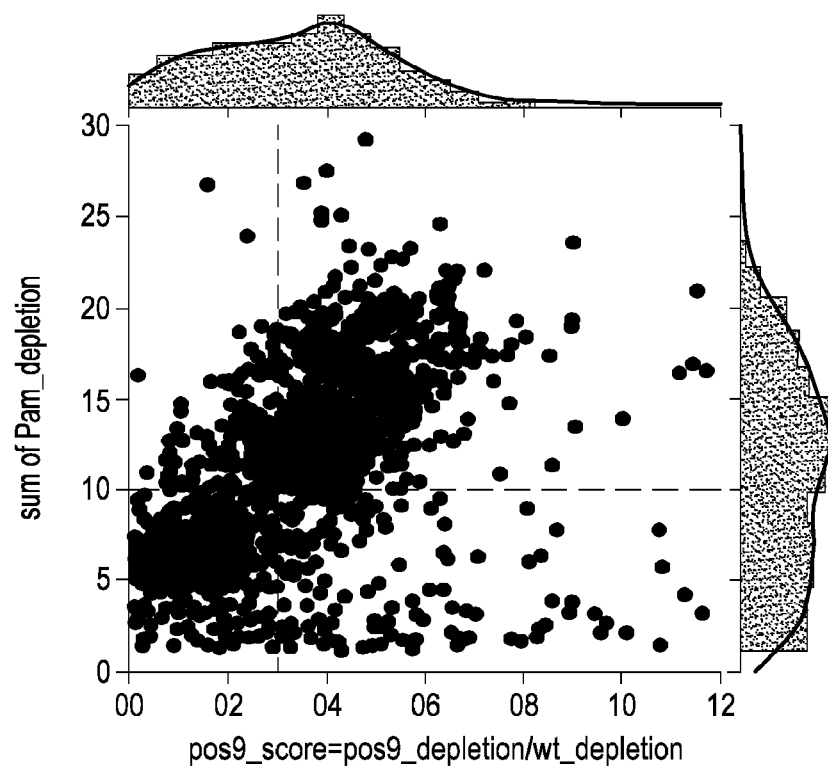
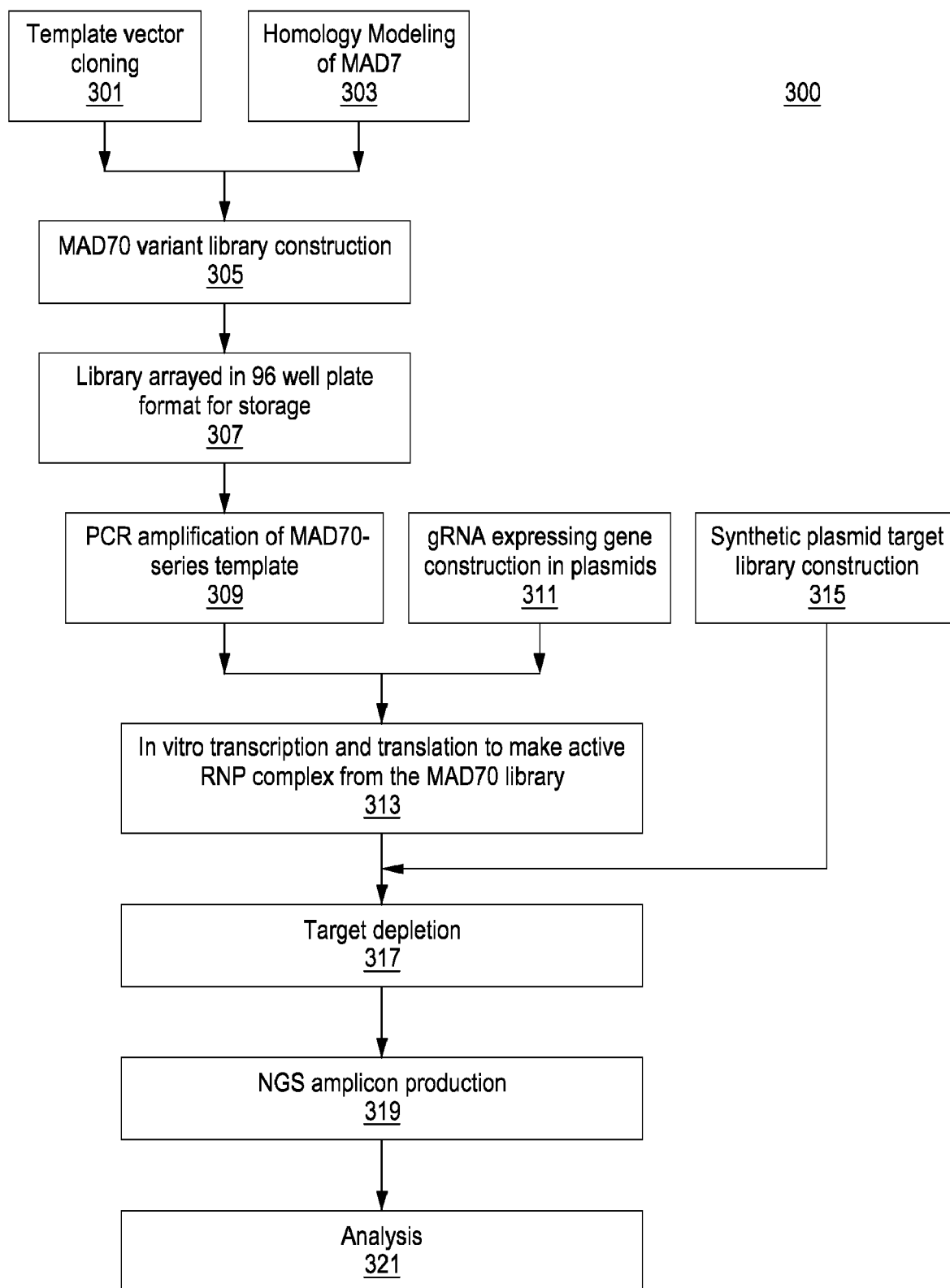


FIG. 2B





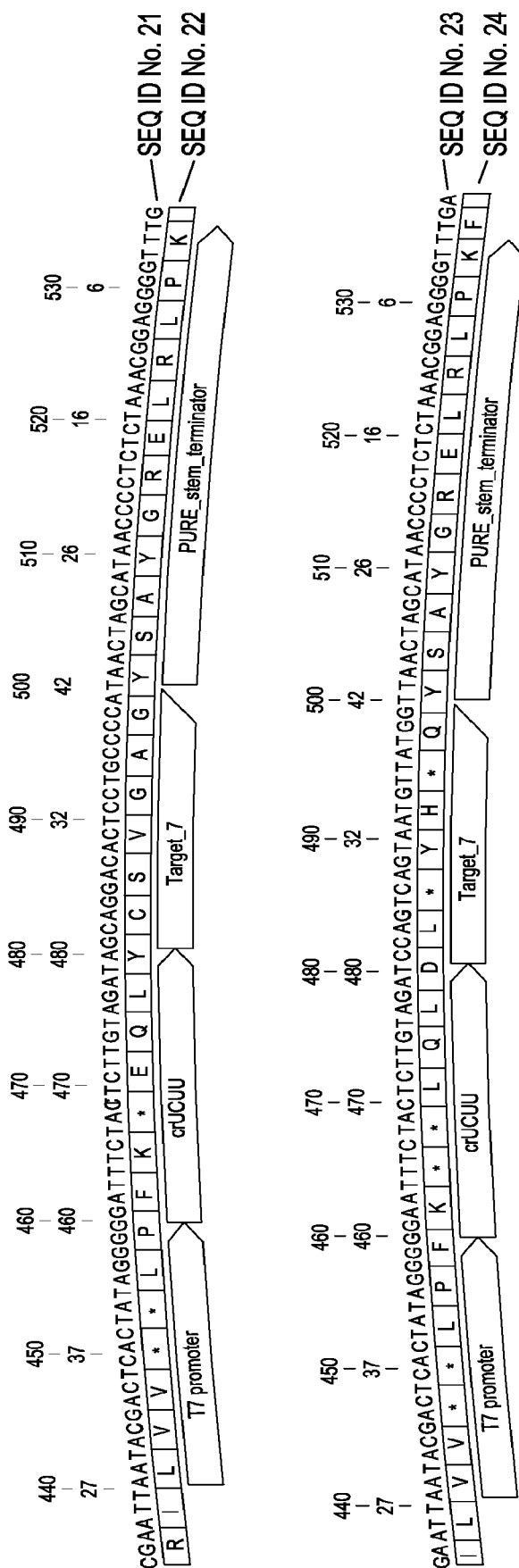


FIG. 4

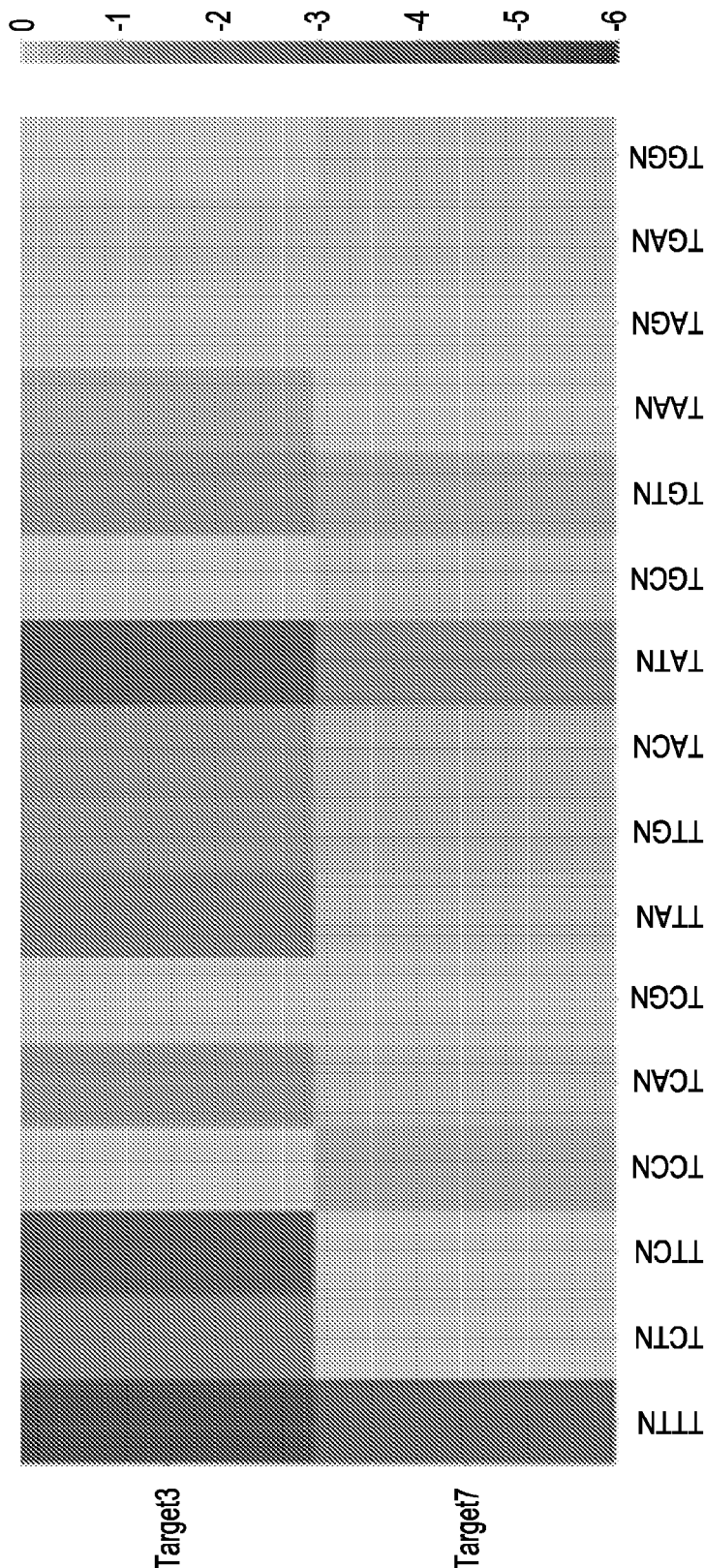


FIG. 5

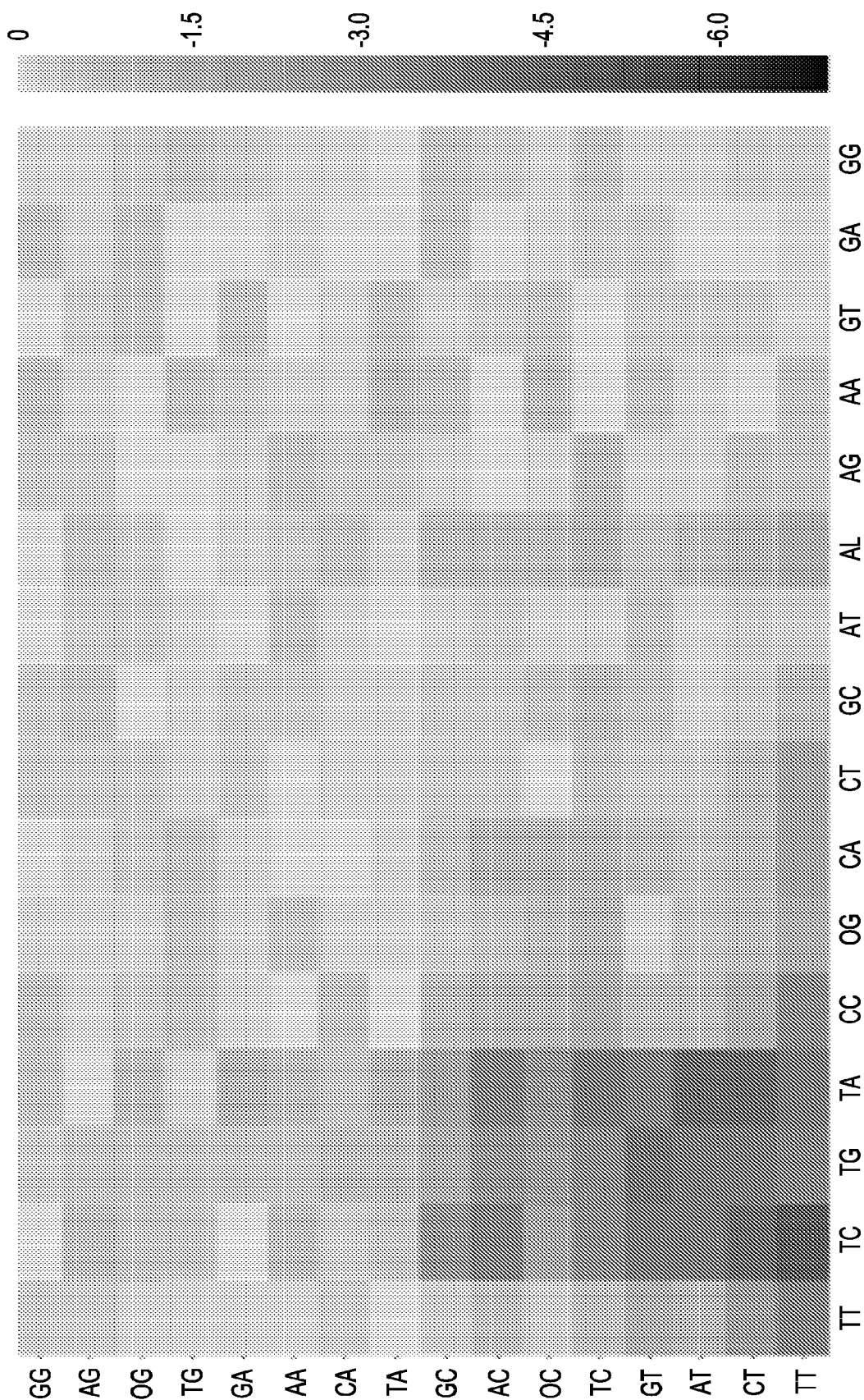


FIG. 6A

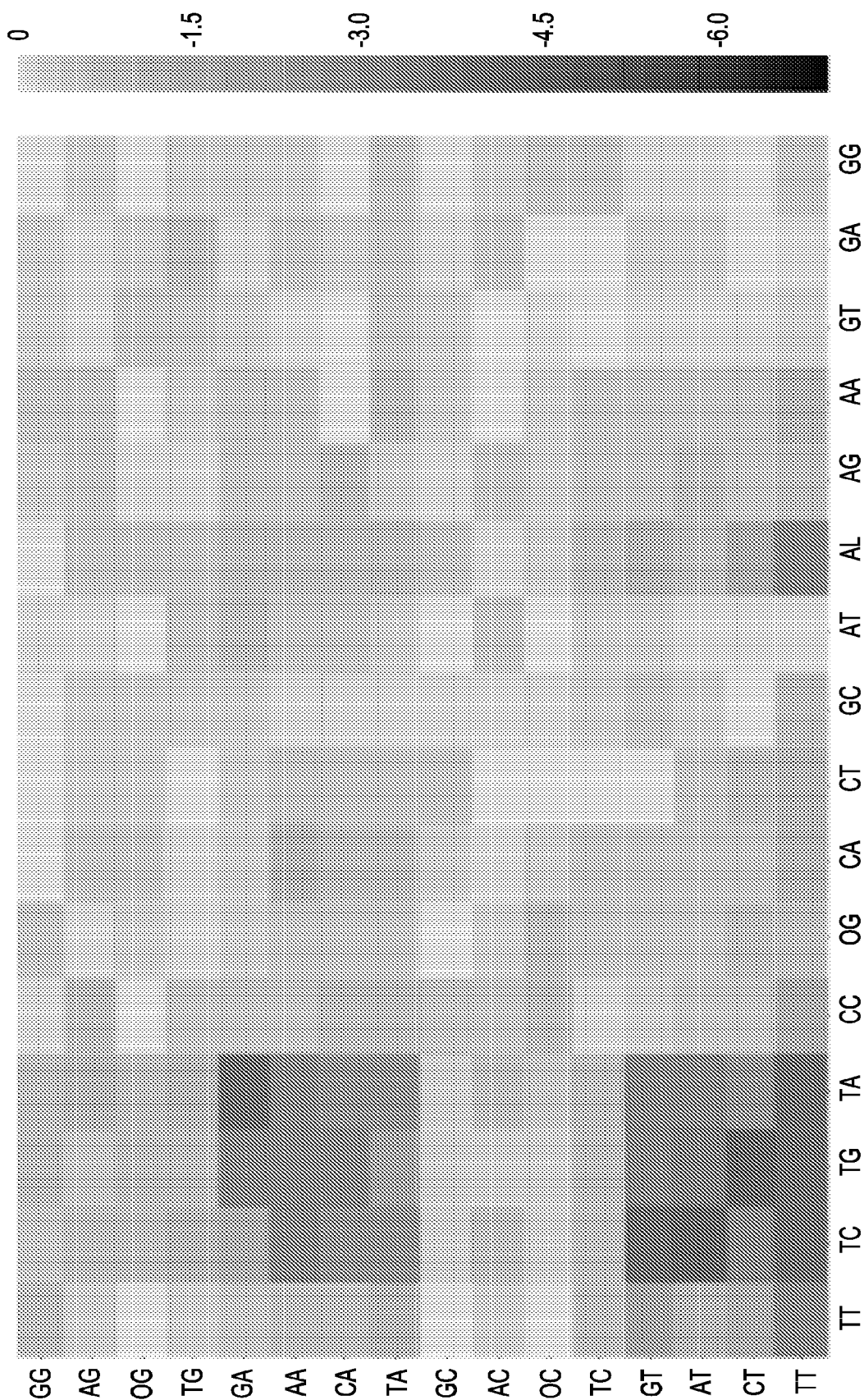


FIG. 6B

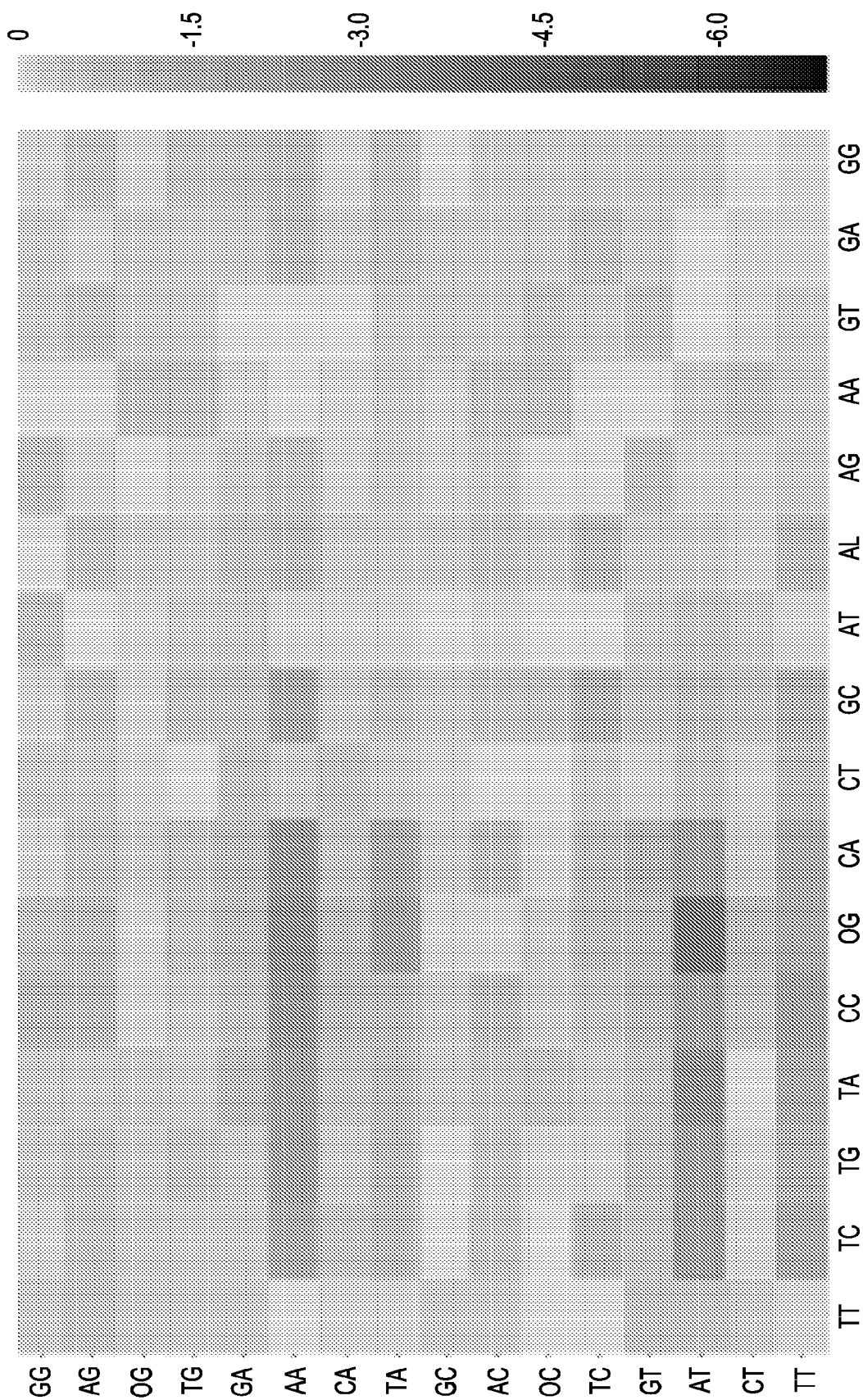


FIG. 6C

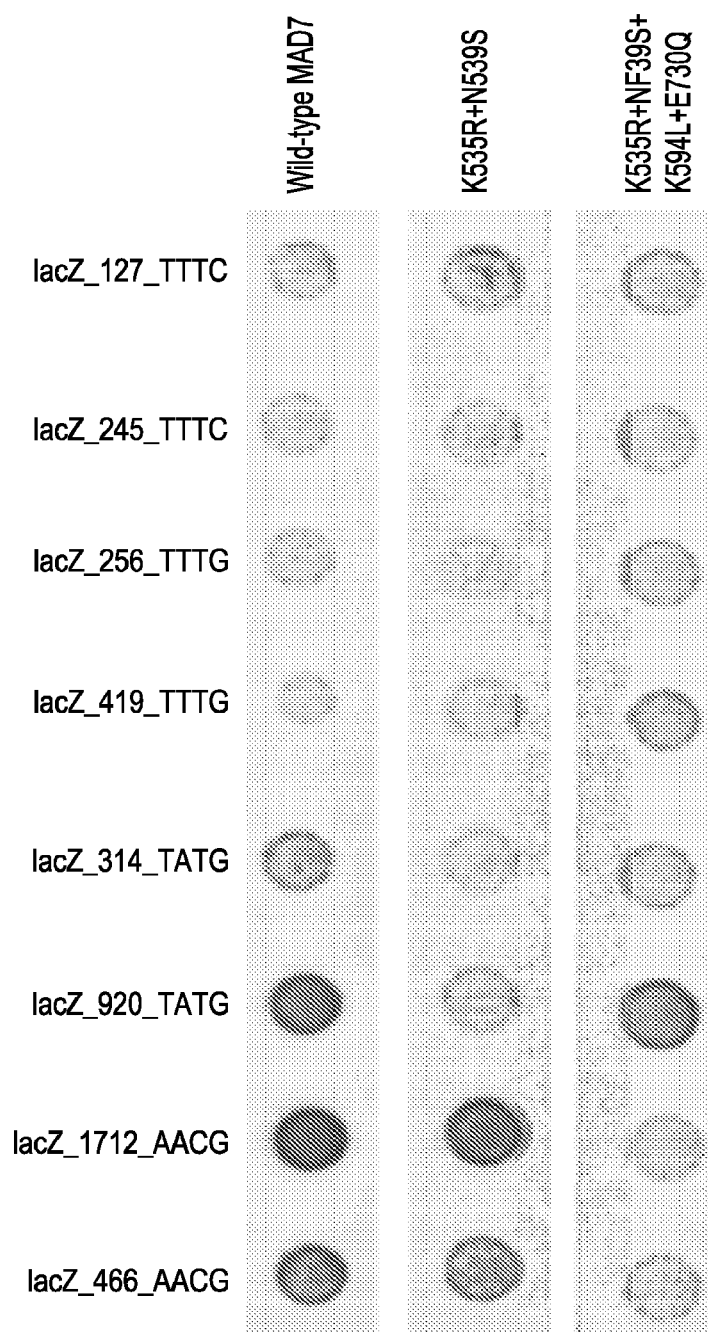


FIG. 7

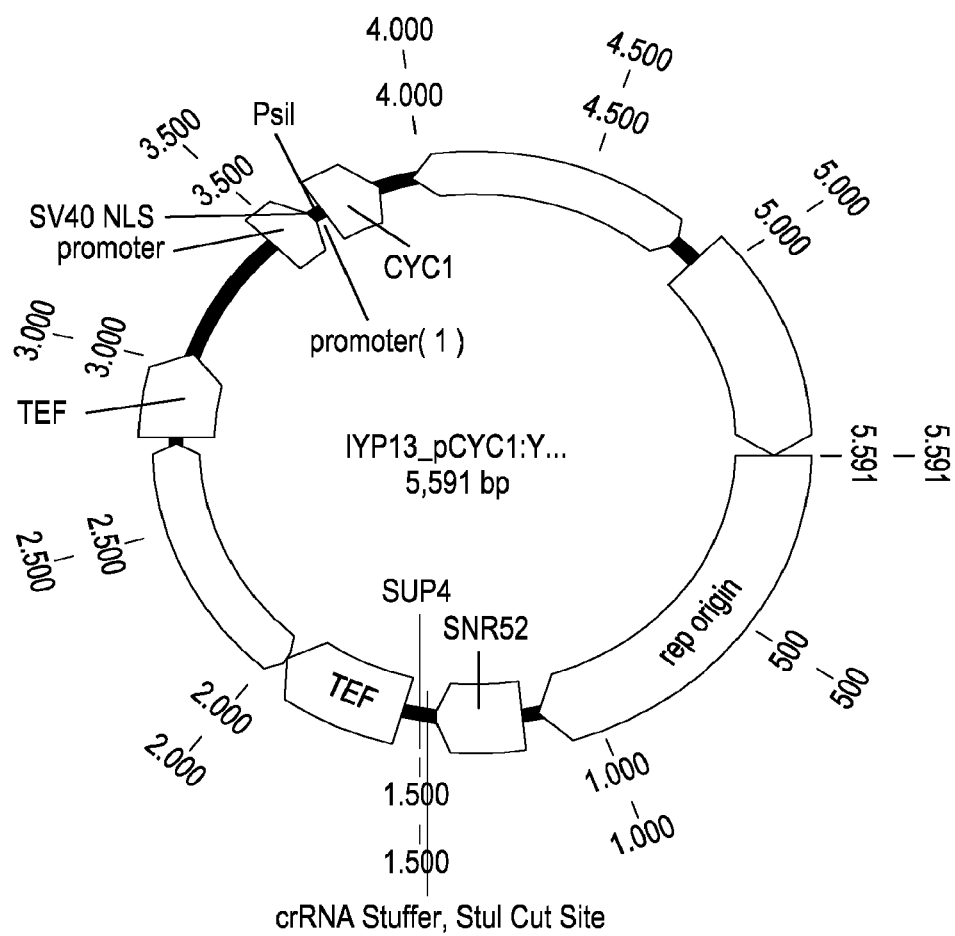


FIG. 8

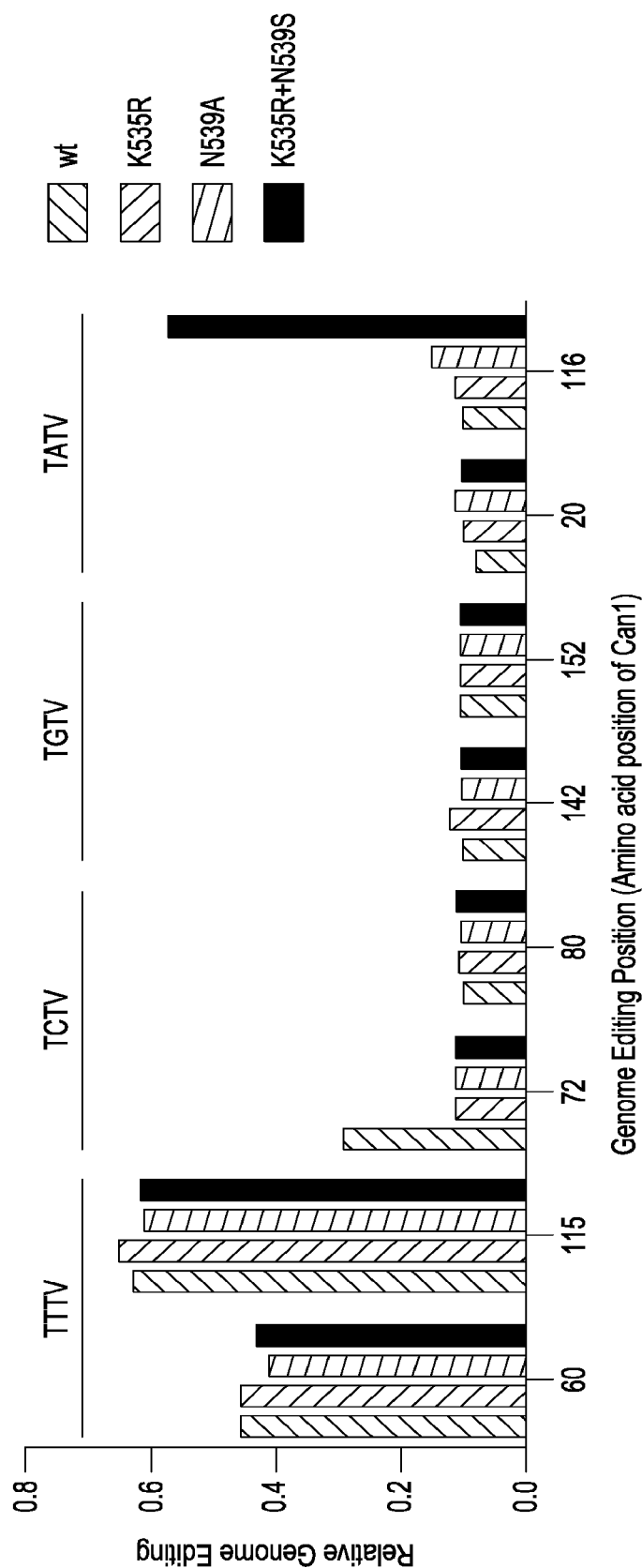


FIG. 9

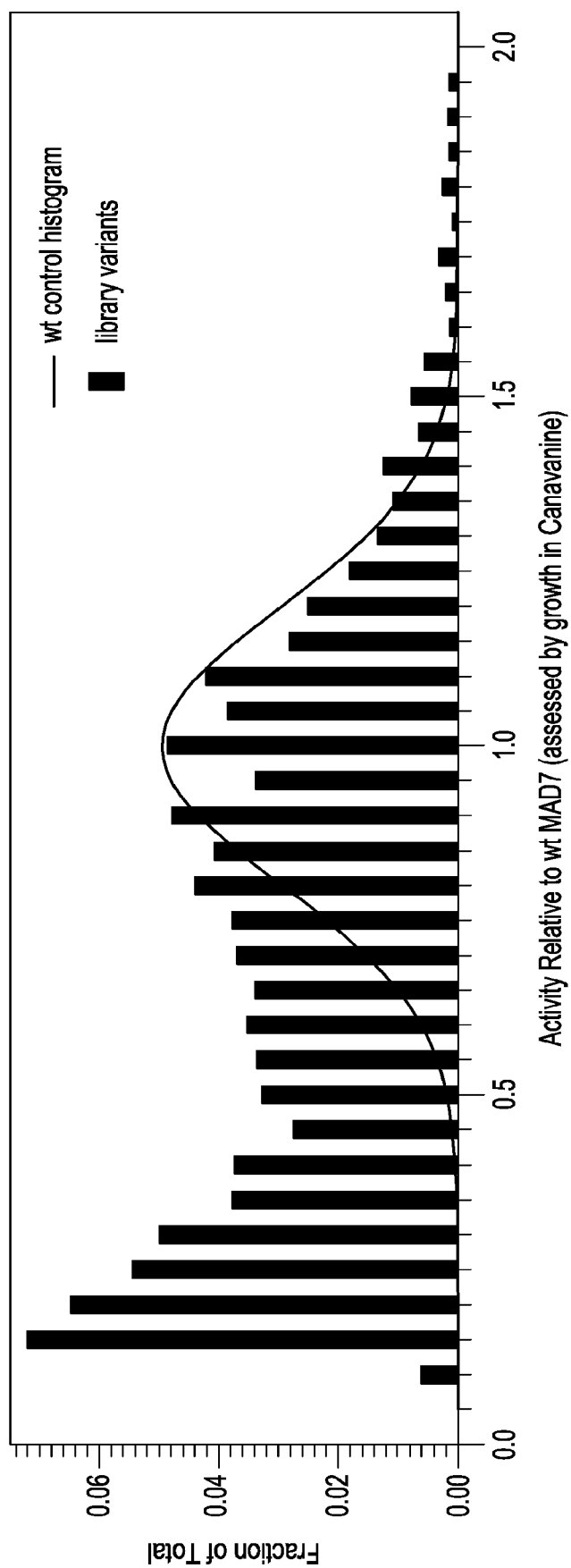


FIG. 10

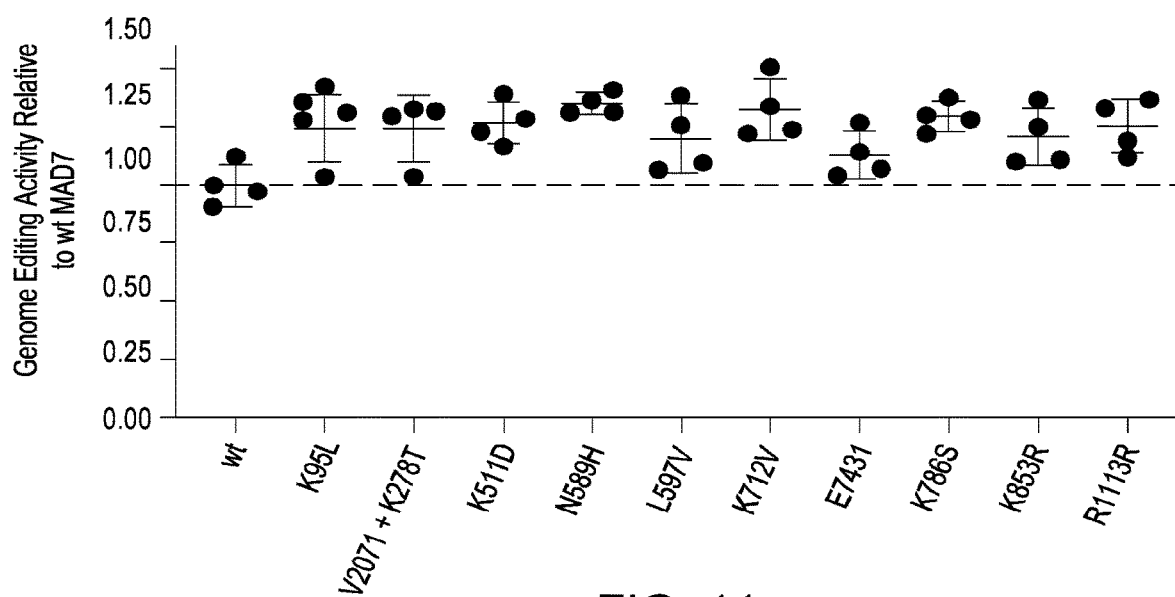


FIG. 11

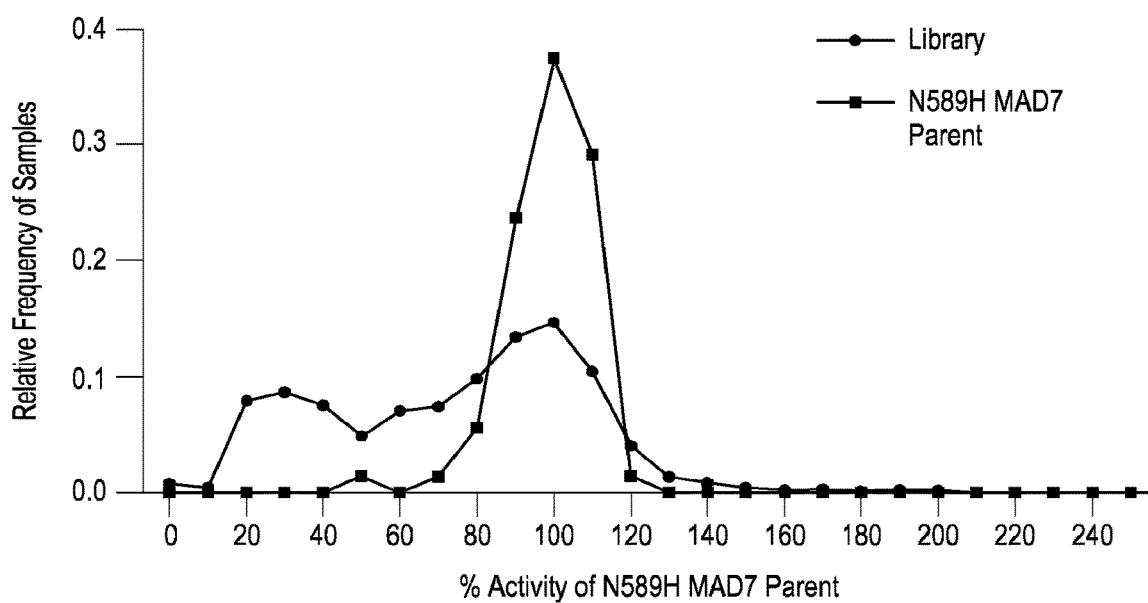


FIG. 12

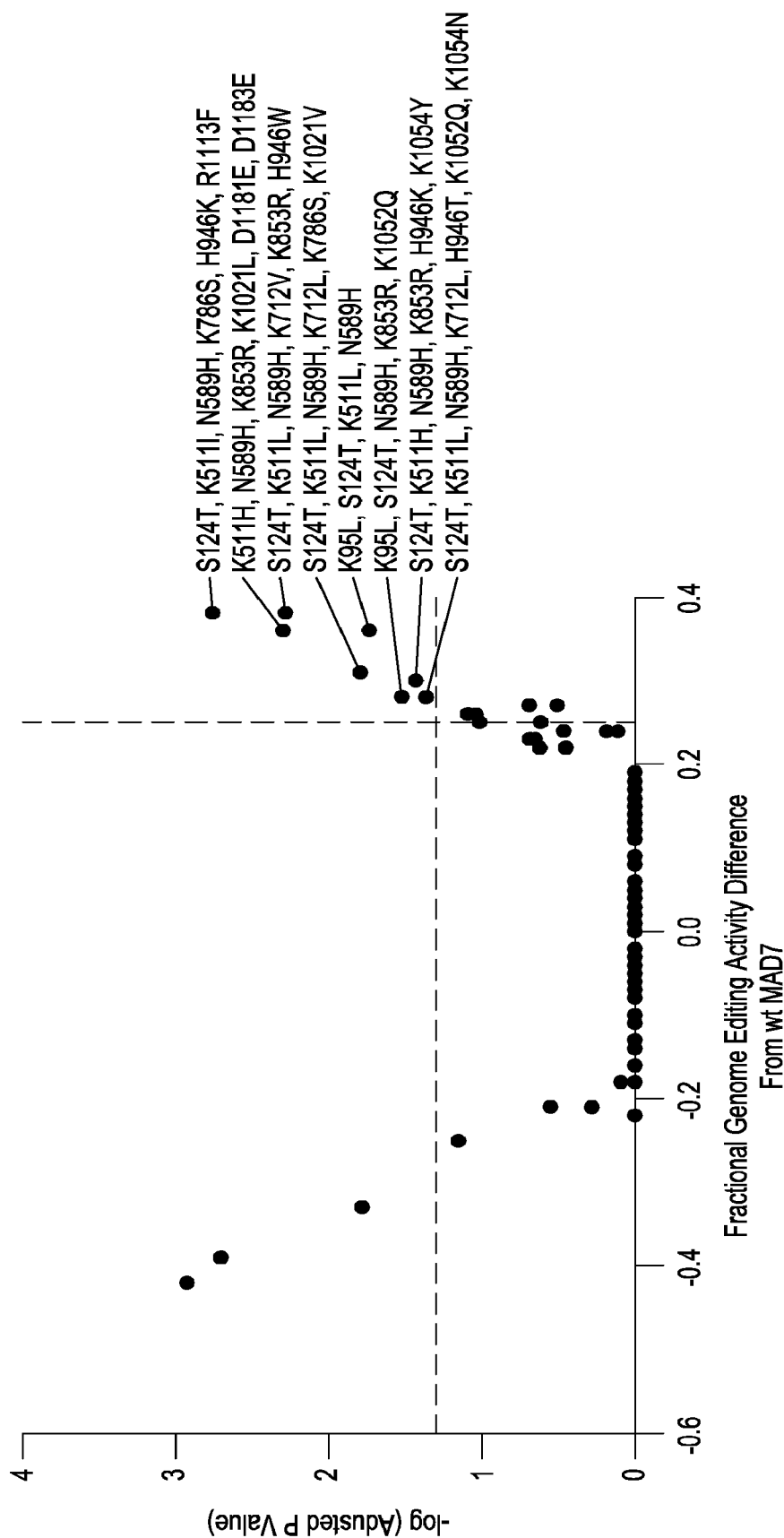


FIG. 13

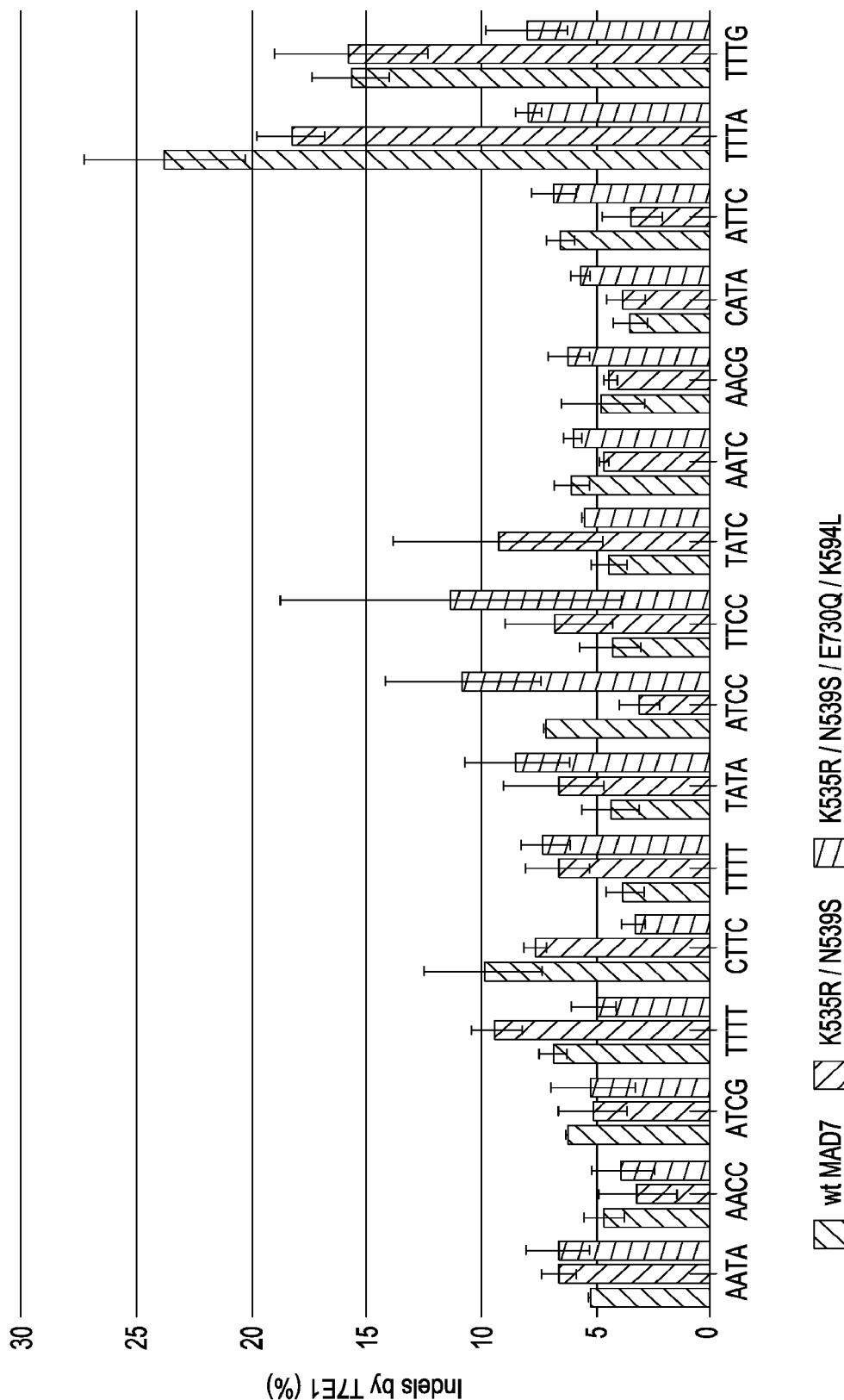


FIG. 14

ENGINEERED ENZYMES**RELATED APPLICATIONS**

This application is a continuation of U.S. Ser. No. 16/844, 079, filed 9 Apr. 2020, now allowed; which is a continuation of U.S. Ser. No. 16/798,315, filed 22 Feb. 2020, now U.S. Pat. No. 10,640,754; which is a continuation of U.S. Ser. No. 16/658,948, filed 21 Oct. 2019, now U.S. Pat. No. 10,604,746; which claims priority to U.S. Provisional Application No. 62/748,668, filed 22 Oct. 2018.

FIELD OF THE INVENTION

This invention relates to engineered enzymes for editing live cells.

BACKGROUND OF THE INVENTION

In the following discussion certain articles and methods will be described for background and introductory purposes. Nothing contained herein is to be construed as an “admission” of prior art. Applicant expressly reserves the right to demonstrate, where appropriate, that the methods referenced herein do not constitute prior art under the applicable statutory provisions.

The ability to make precise, targeted changes to the genome of living cells has been a long-standing goal in biomedical research and development. Recently, various nucleases have been identified that allow manipulation of gene sequence, and hence gene function. These nucleases include nucleic acid-guided nucleases. The range of target sequences that nucleic acid-guided nucleases can recognize, however, is constrained by the need for a specific protospacer adjacent motif (PAM) to be located near the desired target sequence. PAMs are short nucleotide sequences recognized by a gRNA/nuclease complex, where this complex directs editing of a target sequence in a live cell. The precise PAM sequence and length requirements for different nucleic acid-guided nucleases vary; however, PAMs typically are 2-7 base-pair sequences adjacent or in proximity to the target sequence and, depending on the nuclease, can be 5' or 3' to the target sequence. Engineering of nucleic acid-guided nucleases may allow for alteration of PAM preference, allow for editing optimization in different organisms and/or alter enzyme fidelity; all changes that may increase the versatility of a specific nucleic acid-guided nuclease for certain editing tasks.

There is thus a need in the art of nucleic acid-guided nuclease gene editing for improved nucleases. The engineered MAD70-series nucleases described herein satisfy this need.

SUMMARY OF THE INVENTION

This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the following written Detailed Description including those aspects illustrated in the accompanying drawings and defined in the appended claims.

The present disclosure provides engineered MAD70-series nucleases with varied PAM preferences, varied editing

efficiency in different organisms and/or altered RNA-guided enzyme fidelity (e.g., decreased off-target cutting).

Thus, in one embodiment there is provided an engineered MAD70-series nuclease with an altered RNA-guided enzyme fidelity relative to the MAD7 nuclease where the MAD7 nuclease has the amino acid sequence of SEQ ID No. 1. In some aspects of this embodiment, the engineered MAD70-series nuclease with the higher altered RNA-guided enzyme fidelity comprises any of SEQ ID No. 4-7.

In other embodiments there is provided an engineered MAD70-series nuclease having a PAM preference different than the MAD7 nuclease having the sequence of SEQ ID No. 1. In some aspects of this embodiment, the engineered MAD70-series nuclease having an altered PAM preference comprises any of SEQ ID Nos. 2, 3, 11, 12, 13, 14, 67 or 68. In some aspects of this embodiment, there is provided a cocktail of nuclease enzymes comprising one, two, three, four, five or all of SEQ ID Nos. 2, 3, 11, 12, 13, 14, 67 or 68, and in some aspects, there is provided a cocktail of nuclease enzymes comprising one, some or all of SEQ ID Nos. 2, 3, 11, 12, 13, 14, 67 or 68 and another nuclease with a PAM preference different from SEQ ID Nos. 2, 3, 11, 12, 13, 14, 67 or 68, and in some aspects, the other nuclease has a sequence of SEQ ID No. 4, 5, 6, 7, 69-78 or 79-86.

Additionally, there is provided is an engineered MAD70-series nuclease with lower cutting activity relative to the MAD7 nuclease having the sequence of SEQ ID No. 1. In some aspects of this embodiment, the engineered MAD70-series nuclease having lowered cutting activity comprises any of SEQ ID Nos. 8-10 or 15.

Also there is provided an engineered MAD70-series nuclease with enhanced editing efficiency in yeast comprising any of SEQ ID Nos. 69-78 and 79-86.

These aspects and other features and advantages of the invention are described below in more detail.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A is a heatmap for certain of the MAD70-series nucleases with different PAM recognition sites. FIG. 1B-1 is a heatmap for certain of the MAD70-series nucleases with varied fidelity as compared with the MAD7 (SEQ ID No.1). FIG. B-2 shows the PAM depletion panel for the same enzyme from FIG. B-1.

FIGS. 2A and 2B show the results of 108 engineered MAD70-series nucleases selected from screening 1104 single amino acid variants. FIG. 2A is the plot (sum of PAM depletion vs. pos9_score) for the MAD7 nuclease having the sequence SEQ ID NO. 1, and FIG. 2B is the plot for the screened 1104 single amino acid variants.

FIG. 3 is an exemplary workflow for creating and screening engineered MAD70-series enzymes.

FIG. 4 shows the sequence of two different gRNA constructs used for depletion studies (SEQ ID No. 21-24).

FIG. 5 is a heatmap for PAM preferences for MAD70-series variants from a combinatorial library screen.

FIG. 6A is a complete NNNN PAM preference for wild-type MAD 7 (SEQ ID No. 1). FIG. 6B is a complete NNNN PAM preference for the K535R/N539S mutant (SEQ ID No. 67). FIG. 6C is a complete NNNN PAM preference for the K535R/N539S/K594L/E730Q mutant (SEQ ID No. 68).

FIG. 7 shows colonies containing editing cassettes and wild-type MAD7, MAD70-series variants K535R/N539S (SEQ ID No. 67) and K535R/N539S/K594L/E730Q (SEQ ID No. 68) mutants in relation to the wild-type MAD7 amino acid sequence.

FIG. 8 is a map of the plasmid used for the screening of nuclease proteins for genome editing activity in *S. cerevisiae*.

FIG. 9 shows the relative rates of genome editing at different positions of the Can1 protein locus with the indicated PAM by wild-type MAD7, and the K535R (SEQ ID No. 13), N539A and K535R/N539S (SEQ ID No. 67) MAD70-series mutants.

FIG. 10 shows the results of screening 2304 MAD70-series variants for genome editing activity in *S. cerevisiae*.

FIG. 11 shows quadruplicate re-testing of MAD70-series variants that demonstrated enhanced genome editing activity in *S. cerevisiae*.

FIG. 12 shows the results of screening 2304 MAD70-series combinatorial protein variants for genome editing activity in *S. cerevisiae*.

FIG. 13 shows the results of secondary screening of the MAD70-series combinatorial variant hits showing fractional difference in genome editing activity in *S. cerevisiae* and the multiple-comparison-adjusted P value for each variant as compared to the wild-type MAD 7 (SEQ ID No. 1) controls.

FIG. 14 shows the results of genome editing in mammalian HEK293T cells with wild-type MAD7 (SEQ ID No. 1) and MAD70-series variants with AsCas12a as a control.

DETAILED DESCRIPTION

The description set forth below in connection with the appended drawings is intended to be a description of various, illustrative embodiments of the disclosed subject matter. Specific features and functionalities are described in connection with each illustrative embodiment; however, it will be apparent to those skilled in the art that the disclosed embodiments may be practiced without each of those specific features and functionalities. Moreover, all of the functionalities described in connection with one embodiment are intended to be applicable to the additional embodiments described herein except where expressly stated or where the feature or function is incompatible with the additional embodiments. For example, where a given feature or function is expressly described in connection with one embodiment but not expressly mentioned in connection with an alternative embodiment, it should be understood that the feature or function may be deployed, utilized, or implemented in connection with the alternative embodiment unless the feature or function is incompatible with the alternative embodiment.

The practice of the techniques described herein may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, biological emulsion generation, and sequencing technology, which are within the skill of those who practice in the art. Such conventional techniques include polymer array synthesis, hybridization and ligation of polynucleotides, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the examples herein. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Green, et al., Eds. (1999), *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV); Weiner, Gabriel, Stephens, Eds. (2007), *Genetic Variation: A Laboratory Manual*; Dieffenbach, Dveksler, Eds. (2003), *PCR Primer: A Laboratory Manual*; Bowtell and Sambrook (2003), *DNA Microarrays: A Molecular Cloning Manual*; Mount (2004), *Bioinformatics:*

Sequence and Genome Analysis; Sambrook and Russell (2006), *Condensed Protocols from Molecular Cloning: A Laboratory Manual*; and Sambrook and Russell (2002), *Molecular Cloning: A Laboratory Manual* (all from Cold Spring Harbor Laboratory Press); Stryer, L. (1995) *Biochemistry* (4th Ed.) W.H. Freeman, New York N.Y.; Gait, "Oligonucleotide Synthesis: A Practical Approach" 1984, IRL Press, London; Nelson and Cox (2000), *Lehninger, Principles of Biochemistry* 3rd Ed., W. H. Freeman Pub., New York, N.Y.; Berg et al. (2002) *Biochemistry*, 5th Ed., W.H. Freeman Pub., New York, N.Y.; *Cell and Tissue Culture: Laboratory Procedures in Biotechnology* (Doyle & Griffiths, eds., John Wiley & Sons 1998); *Mammalian Chromosome Engineering—Methods and Protocols* (G. Hadlaczy, ed., Humana Press 2011); *Essential Stem Cell Methods*, (Lanza and Klimanskaya, eds., Academic Press 2011), all of which are herein incorporated in their entirety by reference for all purposes. Nuclease-specific techniques can be found in, e.g., *Genome Editing and Engineering From TALENs and CRISPRs to Molecular Surgery*, Appasani and Church, 2018; and *CRISPR: Methods and Protocols*, Lindgren and Charpentier, 2015; both of which are herein incorporated in their entirety by reference for all purposes. Basic methods for enzyme engineering may be found in, *Enzyme Engineering Methods and Protocols*, Samuelson, ed., 2013; *Protein Engineering*, Kaumaya, ed., (2012); and Kaur and Sharma, "Directed Evolution: An Approach to Engineer Enzymes", Crit. Rev. Biotechnology, 26:165-69 (2006).

Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an oligonucleotide" refers to one or more oligonucleotides, and reference to "an automated system" includes reference to equivalent steps and methods for use with the system known to those skilled in the art, and so forth. Additionally, it is to be understood that terms such as "left," "right," "top," "bottom," "front," "rear," "side," "height," "length," "width," "upper," "lower," "interior," "exterior," "inner," "outer" that may be used herein merely describe points of reference and do not necessarily limit embodiments of the present disclosure to any particular orientation or configuration. Furthermore, terms such as "first," "second," "third," etc., merely identify one of a number of portions, components, steps, operations, functions, and/or points of reference as disclosed herein, and likewise do not necessarily limit embodiments of the present disclosure to any particular configuration or orientation.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing devices, methods and cell populations that may be used in connection with the presently described invention.

Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of ordinary skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

The term “complementary” as used herein refers to Watson-Crick base pairing between nucleotides and specifically refers to nucleotides hydrogen bonded to one another with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds. In general, a nucleic acid includes a nucleotide sequence described as having a “percent complementarity” or “percent homology” to a specified second nucleotide sequence. For example, a nucleotide sequence may have 80%, 90%, or 100% complementarity to a specified second nucleotide sequence, indicating that 8 of 10, 9 of 10 or 10 of 10 nucleotides of a sequence are complementary to the specified second nucleotide sequence. For instance, the nucleotide sequence 3'-TCGA-5' is 100% complementary to the nucleotide sequence 5'-AGCT-3'; and the nucleotide sequence 3'-TCGA-5' is 100% complementary to a region of the nucleotide sequence 5'-TTAGCTGG-3'.

The term DNA “control sequences” refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites, nuclear localization sequences, enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these types of control sequences need to be present so long as a selected coding sequence is capable of being replicated, transcribed and—for some components—translated in an appropriate host cell.

As used herein the term “donor DNA” or “donor nucleic acid” refers to nucleic acid that is designed to introduce a DNA sequence modification (insertion, deletion, substitution) into a locus by homologous recombination using nucleic acid-guided nucleases. For homology-directed repair, the donor DNA must have sufficient homology to the regions flanking the “cut site” or site to be edited in the genomic target sequence. The length of the homology arm(s) will depend on, e.g., the type and size of the modification being made. In many instances and preferably, the donor DNA will have two regions of sequence homology (e.g., two homology arms) to the genomic target locus. Preferably, an “insert” region or “DNA sequence modification” region—the nucleic acid modification that one desires to be introduced into a genome target locus in a cell—will be located between two regions of homology. The DNA sequence modification may change one or more bases of the target genomic DNA sequence at one specific site or multiple specific sites. A change may include changing 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 or more base pairs of the target sequence. A deletion or insertion may be a deletion or insertion of 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 150, 200, 300, 400, or 500 or more base pairs of the target sequence.

The terms “guide nucleic acid” or “guide RNA” or “gRNA” refer to a polynucleotide comprising 1) a guide sequence capable of hybridizing to a genomic target locus, and 2) a scaffold sequence capable of interacting or complexing with a nucleic acid-guided nuclease.

“Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or, more often in

the context of the present disclosure, between two nucleic acid molecules. The term “homologous region” or “homology arm” refers to a region on the donor DNA with a certain degree of homology with the target genomic DNA sequence. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences.

“Operably linked” refers to an arrangement of elements where the components so described are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the transcription, and in some cases, the translation, of a coding sequence. The control sequences need not be contiguous with the coding sequence so long as they function to direct the expression of the coding sequence. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered “operably linked” to the coding sequence. In fact, such sequences need not reside on the same contiguous DNA molecule (i.e. chromosome) and may still have interactions resulting in altered regulation.

A “promoter” or “promoter sequence” is a DNA regulatory region capable of binding RNA polymerase and initiating transcription of a polynucleotide or polypeptide coding sequence such as messenger RNA, ribosomal RNA, small nuclear or nucleolar RNA, guide RNA, or any kind of RNA transcribed by any class of any RNA polymerase I, II or III. Promoters may be constitutive or inducible and, in some embodiments—particularly many embodiments in which selection is employed—the transcription of at least one component of the nucleic acid-guided nuclease editing system is under the control of an inducible promoter.

As used herein the term “selectable marker” refers to a gene introduced into a cell, which confers a trait suitable for artificial selection. General use selectable markers are well-known to those of ordinary skill in the art. Drug selectable markers such as ampicillin/carbenicillin, kanamycin, chloramphenicol, erythromycin, tetracycline, gentamicin, bleomycin, streptomycin, rhamnose, puromycin, hygromycin, blasticidin, and G418 may be employed. In other embodiments, selectable markers include, but are not limited to human nerve growth factor receptor (detected with a MAb, such as described in U.S. Pat. No. 6,365,373); truncated human growth factor receptor (detected with MAb); mutant human dihydrofolate reductase (DHFR; fluorescent MTX substrate available); secreted alkaline phosphatase (SEAP; fluorescent substrate available); human thymidylate synthase (TS; confers resistance to anti-cancer agent fluorodeoxyuridine); human glutathione S-transferase alpha (GSTA1; conjugates glutathione to the stem cell selective alkylator busulfan; chemoprotective selectable marker in CD34+ cells); CD24 cell surface antigen in hematopoietic stem cells; human CAD gene to confer resistance to N-phosphonacetyl-L-aspartate (PALA); human multi-drug resistance-1 (MDR-1; P-glycoprotein surface protein selectable by increased drug resistance or enriched by FACS); human CD25 (IL-2a; detectable by Mab-FITC); Methylguanine-DNA methyltransferase (MGMT; selectable by carmustine); and Cytidine deaminase (CD; selectable by Ara-C). “Selective medium” as used herein refers to cell growth medium to which has been added a chemical compound or biological moiety that selects for or against selectable markers.

The terms “target genomic DNA sequence”, “target sequence”, or “genomic target locus” refer to any locus in vitro or in vivo, or in a nucleic acid (e.g., genome) of a cell or population of cells, in which a change of at least one nucleotide is desired using a nucleic acid-guided nuclease editing system. The target sequence can be a genomic locus or extrachromosomal locus.

A “vector” is any of a variety of nucleic acids that comprise a desired sequence or sequences to be delivered to and/or expressed in a cell. Vectors are typically composed of DNA, although RNA vectors are also available. Vectors include, but are not limited to, plasmids, fosmids, phagemids, virus genomes, synthetic chromosomes, and the like. As used herein, the phrase “engine vector” comprises a coding sequence for a nuclease to be used in the nucleic acid-guided nuclease systems and methods of the present disclosure. The engine vector may also comprise, in a bacterial system, the λ Red recombineering system or an equivalent thereto. Engine vectors also typically comprise a selectable marker. As used herein the phrase “editing vector” comprises a donor nucleic acid, optionally including an alteration to the target sequence that prevents nuclease binding at a PAM or spacer in the target sequence after editing has taken place, and a coding sequence for a gRNA. The editing vector may also comprise a selectable marker and/or a barcode. In some embodiments, the engine vector and editing vector may be combined; that is, the contents of the engine vector may be found on the editing vector. Further, the engine and editing vectors comprise control sequences operably linked to, e.g., the nuclease coding sequence, recombineering system coding sequences (if present), donor nucleic acid, guide nucleic acid, and selectable marker(s).

Editing in Nucleic Acid-Guided Nuclease Genome Systems Generally

The present disclosure provides engineered gene editing MAD70-series nucleases with varied PAM preferences, optimized editing efficiency in different organisms, and/or an altered RNA-guided enzyme fidelity. The engineered MAD70-series nucleases may be used to edit all cell types including, archaeal, prokaryotic, and eukaryotic (e.g., yeast, fungal, plant and animal) cells although certain MAD70-series variants exhibit enhanced efficiency in, e.g., yeast or mammalian cells.

The engineered MAD70-series nuclease variants described herein improve RNA-guided enzyme editing systems in which nucleic acid-guided nucleases (e.g., RNA-guided nucleases) are used to edit specific target regions in an organism’s genome. A nucleic acid-guided nuclease complexed with an appropriate synthetic guide nucleic acid in a cell can cut the genome of the cell at a desired location. The guide nucleic acid helps the nucleic acid-guided nuclease recognize and cut the DNA at a specific target sequence. By manipulating the nucleotide sequence of the guide nucleic acid, the nucleic acid-guided nuclease may be programmed to target any DNA sequence for cleavage as long as an appropriate protospacer adjacent motif (PAM) is nearby.

The engineered MAD70-series nucleases may be delivered to cells to be edited as a polypeptide; alternatively, a polynucleotide sequence encoding the engineered MAD70-series nuclease(s) is transformed or transfected into the cells to be edited. The polynucleotide sequence encoding the engineered MAD70-series nuclease may be codon optimized for expression in particular cells, such as archaeal, prokaryotic or eukaryotic cells. Eukaryotic cells can be yeast, fungi, algae, plant, animal, or human cells. Eukaryotic

cells may be those of or derived from a particular organism, such as a mammal, including but not limited to human, mouse, rat, rabbit, dog, or non-human mammals including non-human primates. The choice of the engineered MAD70-series nuclease to be employed depends on many factors, such as what type of edit is to be made in the target sequence and whether an appropriate PAM is located close to the desired target sequence. The engineered MAD70-series nuclease may be encoded by a DNA sequence on a vector (e.g., the engine vector) and be under the control of a constitutive or inducible promoter. In some embodiments, the sequence encoding the nuclease is under the control of an inducible promoter, and the inducible promoter may be separate from but the same as an inducible promoter controlling transcription of the guide nucleic acid; that is, a separate inducible promoter may drive the transcription of the nuclease and guide nucleic acid sequences but the two inducible promoters may be the same type of inducible promoter. Alternatively, the inducible promoter controlling expression of the nuclease may be different from the inducible promoter controlling transcription of the guide nucleic acid.

In general, a guide nucleic acid (e.g., gRNA) complexes with a compatible nucleic acid-guided nuclease and can then hybridize with a target sequence, thereby directing the nuclease to the target sequence. In certain aspects, the RNA-guided enzyme editing system may use two separate guide nucleic acid molecules that combine to function as a guide nucleic acid, e.g., a CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). In other aspects—and used with the MAD70-series variant nucleases described herein—the guide nucleic acid may be a single guide nucleic acid that includes both the crRNA and tracrRNA sequences. A guide nucleic acid can be DNA or RNA; alternatively, a guide nucleic acid may comprise both DNA and RNA. In some embodiments, a guide nucleic acid may comprise modified or non-naturally occurring nucleotides. In cases where the guide nucleic acid comprises RNA, the gRNA may be encoded by a DNA sequence on a polynucleotide molecule such as a plasmid, linear construct, or the coding sequence may reside within an editing cassette and is under the control of a constitutive promoter, or, in some embodiments, an inducible promoter as described below.

A guide nucleic acid comprises a guide sequence, where the guide sequence is a polynucleotide sequence having sufficient complementarity with a target sequence to hybridize with the target sequence and direct sequence-specific binding of a complexed nucleic acid-guided nuclease to the target sequence. The degree of complementarity between a guide sequence and the corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences. In some embodiments, a guide sequence is about or more than about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20 nucleotides in length. Preferably the guide sequence is 10-30 or 15-20 nucleotides long, or 15, 16, 17, 18, 19, or 20 nucleotides in length.

In the present methods and compositions, the guide nucleic acid typically is provided as a sequence to be expressed from a plasmid or vector and comprises both the guide sequence and the scaffold sequence as a single tran-

script under the control of a promoter, and in some embodiments, an inducible promoter. The guide nucleic acid can be engineered to target a desired target sequence by altering the guide sequence so that the guide sequence is complementary to a desired target sequence, thereby allowing hybridization between the guide sequence and the target sequence. In general, to generate an edit in the target sequence, the gRNA/nuclease complex binds to a target sequence as determined by the guide RNA, and the nuclease recognizes a photospacer adjacent motif (TAM) sequence adjacent to the target sequence. The target sequence can be any polynucleotide endogenous or exogenous to a prokaryotic or eukaryotic cell, or in vitro. For example, the target sequence can be a polynucleotide residing in the nucleus of a eukaryotic cell. A target sequence can be a sequence encoding a gene product (e.g., a protein) or a non-coding sequence (e.g., a regulatory polynucleotide, an intron, a PAM, or “junk” DNA).

The guide nucleic acid may be part of an editing cassette that encodes the donor nucleic acid, such as described in U.S. Pat. No. 10,240,167, issued 26 Mar. 2019; U.S. Pat. No. 10,266,849, issued 23 Apr. 2019; U.S. Pat. No. 9,982,278, issued 22 Jun. 2018; U.S. Pat. No. 10,351,877, issued 15 Jul. 2019; and U.S. Pat. No. 10,362,422, issued 30 Jul. 2019; and U.S. Ser. No. 16/275,439, filed 14 Feb. 2019; Ser. No. 16/275,465, filed 14 Feb. 2019; Ser. No. 16/550,092, filed 23 Aug. 2019; and Ser. No. 16/552,517, filed 26 Aug. 2019. Alternatively, the guide nucleic acid may not be part of the editing cassette and instead may be encoded on the engine or editing vector backbone. For example, a sequence coding for a guide nucleic acid can be assembled or inserted into a vector backbone first, followed by insertion of the donor nucleic acid in, e.g., the editing cassette. In other cases, the donor nucleic acid in, e.g., an editing cassette can be inserted or assembled into a vector backbone first, followed by insertion of the sequence coding for the guide nucleic acid. In yet other cases, the sequence encoding the guide nucleic acid and the donor nucleic acid (inserted, for example, in an editing cassette) are simultaneously but separately inserted or assembled into a vector. In yet other embodiments, the sequence encoding the guide nucleic acid and the sequence encoding the donor nucleic acid are both included in the editing cassette.

The target sequence is associated with a PAM, which is a short nucleotide sequence recognized by the gRNA/nuclease complex. The precise PAM sequence and length requirements for different nucleic acid-guided nucleases vary; however, PAMs typically are 2-7 base-pair sequences adjacent or in proximity to the target sequence and, depending on the nuclease, can be 5' or 3' to the target sequence. Engineering of the PAM-interacting domain of a nucleic acid-guided nuclease may allow for alteration of PAM specificity, improve fidelity, or decrease fidelity. In certain embodiments, the genome editing of a target sequence both introduces a desired DNA change to a target sequence, e.g., the genomic DNA of a cell, and removes, mutates, or renders inactive a proto-spacer mutation (PAM) region in the target sequence. Rendering the PAM at the target sequence inactive precludes additional editing of the cell genome at that target sequence, e.g., upon subsequent exposure to a nucleic acid-guided nuclease complexed with a synthetic guide nucleic acid in later rounds of editing. Thus, cells having the desired target sequence edit and an altered PAM can be selected using a nucleic acid-guided nuclease complexed with a synthetic guide nucleic acid complementary to the target sequence. Cells that did not undergo the first editing event will be cut rendering a double-stranded DNA break,

and thus will not continue to be viable. The cells containing the desired target sequence edit and PAM alteration will not be cut, as these edited cells no longer contain the necessary PAM site and will continue to grow and propagate.

The range of target sequences that nucleic acid-guided nucleases can recognize is constrained by the need for a specific PAM to be located near the desired target sequence. As a result, it often can be difficult to target edits with the precision that is necessary for genome editing. It has been found that nucleases can recognize some PAMs very well (e.g., canonical PAMs), and other PAMs less well or poorly (e.g., non-canonical PAMs). Because certain of the engineered MAD70-series nucleases disclosed herein recognize different PAMs, the engineered MAD70-series nucleases increase the number of target sequences that can be targeted for editing; that is, engineered MAD70-series nucleases decrease the regions of “PAM deserts” in the genome. Thus, the engineered MAD70-series nucleases expand the scope of target sequences that may be edited by increasing the number (variety) of PAM sequences recognized. Moreover, cocktails of engineered MAD70-series nucleases may be delivered to cells such that target sequences adjacent to several different PAMs may be edited in a single editing run.

Another component of the nucleic acid-guided nuclease system is the donor nucleic acid. In some embodiments, the donor nucleic acid is on the same polynucleotide (e.g., editing vector or editing cassette) as the guide nucleic acid and may be (but not necessarily) under the control of the same promoter as the guide nucleic acid (e.g., a single promoter driving the transcription of both the guide nucleic acid and the donor nucleic acid). The donor nucleic acid is designed to serve as a template for homologous recombination with a target sequence nicked or cleaved by the nucleic acid-guided nuclease as a part of the gRNA/nuclease complex. A donor nucleic acid polynucleotide may be of any suitable length, such as about or more than about 20, 25, 50, 75, 100, 150, 200, 500, or 1000 nucleotides in length. In certain preferred aspects, the donor nucleic acid can be provided as an oligonucleotide of between 20-300 nucleotides, more preferably between 50-250 nucleotides. The donor nucleic acid comprises a region that is complementary to a portion of the target sequence (e.g., a homology arm). When optimally aligned, the donor nucleic acid overlaps with (is complementary to) the target sequence by, e.g., about 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 or more nucleotides. In many embodiments, the donor nucleic acid comprises two homology arms (regions complementary to the target sequence) flanking the mutation or difference between the donor nucleic acid and the target template. The donor nucleic acid comprises at least one mutation or alteration compared to the target sequence, such as an insertion, deletion, modification, or any combination thereof compared to the target sequence.

As mentioned previously, often the donor nucleic acid is provided as an editing cassette, which is inserted into a vector backbone where the vector backbone may comprise a promoter driving transcription of the gRNA and the coding sequence of the gRNA, or the vector backbone may comprise a promoter driving the transcription of the gRNA but not the gRNA itself. Moreover, there may be more than one, e.g., two, three, four, or more guide nucleic acid/donor nucleic acid cassettes inserted into an engine vector, where each guide nucleic acid is under the control of separate different promoters, separate like promoters, or where all guide nucleic acid/donor nucleic acid pairs are under the control of a single promoter. In some embodiments—such as embodiments where cell selection is employed—the pro-

motor driving transcription of the gRNA and the donor nucleic acid (or driving more than one gRNA/donor nucleic acid pair) is an inducible promoter. Inducible editing is advantageous in that singulated cells can be grown for several to many cell doublings before editing is initiated, which increases the likelihood that cells with edits will survive, as the double-strand cuts caused by active editing are largely toxic to the cells. This toxicity results both in cell death in the edited colonies, as well as a lag in growth for the edited cells that do survive but must repair and recover following editing. However, once the edited cells have a chance to recover, the size of the colonies of the edited cells will eventually catch up to the size of the colonies of unedited cells. See, e.g., U.S. Ser. No. 16/399,988, filed 30 Apr. 2019; Ser. No. 16/454,865 filed 26 Jun. 2019; and Ser. No. 16/540,606, filed 14 Aug. 2019. Further, a guide nucleic acid may be efficacious directing the edit of more than one donor nucleic acid in an editing cassette; e.g., if the desired edits are close to one another in a target sequence.

In addition to the donor nucleic acid, an editing cassette may comprise one or more primer sites. The primer sites can be used to amplify the editing cassette by using oligonucleotide primers; for example, if the primer sites flank one or more of the other components of the editing cassette.

Also, as described above, the donor nucleic acid may comprise—in addition to the at least one mutation relative to a target sequence—one or more PAM sequence alterations that mutate, delete or render inactive the PAM site in the target sequence. The PAM sequence alteration in the target sequence renders the PAM site “immune” to the nucleic acid-guided nuclease and protects the target sequence from further editing in subsequent rounds of editing if the same nuclease is used.

In addition, the editing cassette may comprise a barcode. A barcode is a unique DNA sequence that corresponds to the donor DNA sequence such that the barcode can identify the edit made to the corresponding target sequence. The barcode typically comprises four or more nucleotides. In some embodiments, the editing cassettes comprise a collection of donor nucleic acids representing, e.g., gene-wide or genome-wide libraries of donor nucleic acids. The library of editing cassettes is cloned into vector backbones where, e.g., each different donor nucleic acid is associated with a different barcode.

Additionally, in some embodiments, an expression vector or cassette encoding components of the nucleic acid-guided nuclease system further encodes an engineered MAD70-series nuclease comprising one or more nuclear localization sequences (NLSs), such as about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs. In some embodiments, the engineered nuclease comprises NLSs at or near the amino-terminus, NLSs at or near the carboxy-terminus, or a combination.

The engine and editing vectors comprise control sequences operably linked to the component sequences to be transcribed. As stated above, the promoters driving transcription of one or more components of the engineered MAD70-series nuclease editing system may be inducible, and an inducible system is likely employed if selection is to be performed. A number of gene regulation control systems have been developed for the controlled expression of genes in plant, microbe, and animal cells, including mammalian cells, including the pL promoter (induced by heat inactivation of the CI857 repressor), the pBAD promoter (induced by the addition of arabinose to the cell growth medium), and the rhamnose inducible promoter (induced by the addition of rhamnose to the cell growth medium). Other systems

include the tetracycline-controlled transcriptional activation system (Tet-On/Tet-Off, Clontech, Inc. (Palo Alto, Calif.)); Bujard and Gossen, PNAS, 89(12):5547-5551 (1992)), the Lac Switch Inducible system (Wyborski et al., Environ Mol Mutagen, 28(4):447-58 (1996); DuCoeur et al., Strategies 5(3):70-72 (1992); U.S. Pat. No. 4,833,080), the ecdysone-inducible gene expression system (No et al., PNAS, 93(8):3346-3351 (1996)), the cumate gene-switch system (Mullick et al., BMC Biotechnology, 6:43 (2006)), and the tamoxifen-inducible gene expression (Zhang et al., Nucleic Acids Research, 24:543-548 (1996)) as well as others.

Typically, performing genome editing in live cells entails transforming cells with the components necessary to perform nucleic acid-guided nuclease editing. For example, the cells may be transformed simultaneously with separate engine and editing vectors; the cells may already be expressing the engineered MAD70-series nuclease (e.g., the cells may have already been transformed with an engine vector or the coding sequence for the engineered MAD70-series nuclease may be stably integrated into the cellular genome) such that only the editing vector needs to be transformed into the cells; or the cells may be transformed with a single vector comprising all components required to perform nucleic acid-guided nuclease genome editing.

A variety of delivery systems can be used to introduce (e.g., transform or transfect) nucleic acid-guided nuclease editing system components into a host cell. These delivery systems include the use of yeast systems, lipofection systems, microinjection systems, biolistic systems, virosomes, liposomes, immunoliposomes, polycations, lipid:nucleic acid conjugates, virions, artificial virions, viral vectors, electroporation, cell permeable peptides, nanoparticles, nanowires, exosomes. Alternatively, molecular trojan horse liposomes may be used to deliver nucleic acid-guided nuclease components across the blood brain barrier. Of particular interest is the use of electroporation, particularly flow-through electroporation (either as a stand-alone instrument or as a module in an automated multi-module system) as described in, e.g., U.S. Pat. No. 10,435,717, issued 8 Oct. 2019; and U.S. Pat. No. 10,443,074, issued 15 Oct. 2019; U.S. Ser. No. 16/550,790, filed 26 Aug. 2019; Ser. No. 10/323,258, issued 18 Jun. 2019; and Ser. No. 10/415,058, issued 17 Sep. 2019.

After the cells are transformed with the components necessary to perform nucleic acid-guided nuclease editing, the cells are cultured under conditions that promote editing. For example, if constitutive promoters are used to drive transcription of the engineered MAD70-series nucleases and/or gRNA, the transformed cells need only be cultured in a typical culture medium under typical conditions (e.g., temperature, CO₂ atmosphere, etc.) Alternatively, if editing is inducible—by, e.g., activating inducible promoters that control transcription of one or more of the components needed for nucleic acid-guided nuclease editing, such as, e.g., transcription of the gRNA, donor DNA, nuclease, or, in the case of bacteria, a recombinering system—the cells are subjected to inducing conditions. The MAD70 nucleases described herein may be used in automated systems, such as those described in U.S. Pat. No. 10,253,316, issued 9 Apr. 2019; U.S. Pat. No. 10,329,559, issued 25 Jun. 2019; U.S. Pat. No. 10,323,242, issued 18 Jun. 2019; and U.S. Pat. No. 10,421,959, issued 24 Sep. 2019; and U.S. Ser. No. 16/412,195, filed 14 May 2019; Ser. No. 16/423,289, filed 28 May 2019; and Ser. No. 16/571,091, filed 14 Sep. 2019.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure

13

and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention, nor are they intended to represent or imply that the experiments below are all of or the only experiments performed. It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific aspects without departing from the spirit or scope of the invention as broadly described. The present aspects are, therefore, to be considered in all respects as illustrative and not restrictive.

Example 1: Exemplary Workflow Overview

FIG. 3 shows an exemplary workflow 300 for creating and screening engineered MAD70-series enzymes. In a first step 301, a wild type MAD7 DNA sequence was prepared and cloned to make a template vector for creation of MAD70-series variants. In another step 303, computer homology modeling of MAD7 (represented by an amino acid sequence having the sequence SEQ ID No. 1) was performed to identify putative regions of interest for rationally-designing MAD70 variants with varied PAM preferences, optimized activity in specific organisms, and altered fidelity as compared to MAD7. These regions include regions of the nuclease proximal to key regions where it is predicted that the nuclease interacts with the PAM, target, or gRNA e.g., see Example 2 below. Once putative key regions of interest were identified in silico, cassettes were constructed and cloned into the vector template, then transformed into cells 305, thereby generating a library of engineered MAD70-series variants. The cells transformed with the engineered MAD70-series variants were arrayed in 96-well plates 307 for storage. At step 309, an aliquot of the cells from each well was taken, and the MAD70-series sequences were amplified from each aliquot. At another step 311, a plasmid expressing a gRNA was constructed, and then combined with the amplified MAD70-series nucleases to perform in vitro transcription and translation to make active ribonuclease protein complexes 313. A synthetic target library was constructed 315, in which to test target depletion 317 for each of that MAD70-series variants. After target depletion, amplicons were produced for analysis using next-gen sequencing 319, and sequencing data analysis was performed 321 to determine target depletion.

Example 2: Homology Modeling and Positions for Mutation Testing

An in silico homology model of a MAD7 enzyme having the amino acid sequence as represented by SEQ ID No. 1 was made using PDB:5B43 structure as a template using SWISS-MODEL (<https://swissmodel.expasy.org>). Mutation sets were generated based on residue proximity to putative key regions of where the nuclease is predicted to interact with the PAM site, target, or gRNA, as well as targeting charged amino acids. The following amino acid residues were targeted for mutation (the residues are in relation to the MAD7 amino acid sequence in SEQ ID No. 1): 19, 22, 55, 84, 95, 124, 125, 159, 160, 161, 162, 165, 169, 171, 187, 269, 278, 281, 283, 284, 346, 466, 505, 511, 517, 528, 529, 530, 531, 532, 533, 534, 535, 536, 539, 582, 584, 586, 587, 588, 589, 590, 591, 593, 594, 595, 596, 597, 598, 599, 600, 601, 620, 623, 650, 707, 712, 720, 739, 741, 742, 743, 749, 761, 768, 785, 786, 822, 830, 833, 842, 853, 878, 881, 912,

14

920, 924, 925, 932, 934, 937, 946, 969, 970, 974, 982, 990, 997, 1019, 1021, 1052, 1054, 1109, 1111, 1113, 1173.

Example 3: Vector Cloning, MAD70-Series Variant Library Construction and PCR

The MAD7 coding sequence was cloned into a pUC57 vector with T7-promoter sequence attached to the 5'-end of the coding sequence and a T7-terminator sequence attached to the 3'-end of the coding sequence. Next, using a pUC57-MAD7 wildtype vector as a template, a saturated mutation library for the 96 positions predicted by the modeling described in Example 2 was made substituting the original codon with NNK (IUPAC code for DNA: N=A, T, G, C; K=G, T) randomized codons. The engineered MAD70-series variants were delivered as a pool of mutant plasmids. 100 ng of a plasmid mixture was transformed into five E.coli® SUPREME electrocompetent solo cells (Lucigen). After the cells were recovered in 5 mL of recovery medium at 37° C. for 1 hr in a shaking incubator, 1 mL of 50% glycerol was added and the cells were stored at -80° C. as 100 µL aliquots.

The stored cells were diluted in phosphate buffered saline and spread on LB agar plates with 100 µg/mL of carbenicillin. The cells were then grown overnight at 37° C. in an incubator. Colonies were picked and inoculated into 1 mL of LB medium (100 µg/mL of carbenicillin) in 96-well culture blocks. Cultures were grown overnight in a shaking incubator at 37° C. Next, 1 µL of the cells were diluted into 500 µL of PCR grade water, and 25 µL aliquots of diluted cultures were boiled for 5 min at 95° C. using a thermal cycler. The boiled cells were used to PCR amplify the different engineered MAD70-series variant coding sequences. The rest of the cultures were stored at -80° C. with added glycerol at 10% v/v concentration.

First, Q5 Hot Start 2× master mix reagent (NEB) was used to amplify the engineered MAD70-series variant sequences using the boiled cells as a source of MAD70-series variant templates. The forward primer 5'-TTGGGTAACGCCAGGGTTTT (SEQ ID No. 16) and reverse primer 5'-TGTGTGGAATTGTGAGCGGA (SEQ ID No. 17) amplified the sequences flanking the engineered MAD70-series variant in the pUC57 vector including the T7-promoter and T7-terminator components attached to the MAD7 variant sequence at the 5'- and 3'-end of the engineered MAD70-series variants, respectively. 1 µM primers were used in a 10 µL PCR reaction using 3.3 µL boiled cell samples as templates in 96 well PCR plates. The PCR conditions shown in Table 1 were used:

TABLE 1

PCR conditions		
STEP	TEMPERATURE	TIME
DENATURATION	98° C.	30 SEC
30 CYCLES	98° C.	10 SEC
	66° C.	30 SEC
	72° C.	2.5 MIN
FINAL EXTENSION	72° C.	2 MIN
HOLD	12° C.	

Example 4: gRNA Expression Gene Construction in Plasmids and Synthetic Target Library Construction

Two plasmids were made to produce two different guide RNAs for the in vitro depletion assay. A MAD7 gRNA

15

scaffold sequence (5'-GGAATTCTACTCTTGTAGAT (SEQ ID No. 18)) was placed under the control of the T7 promoter followed by a guide sequence for synthetic Target 3 or Target 7. The sequences of these constructs are shown in FIG. 4.

Two different synthetic target sequences were used to design a synthetic plasmid target library, where the target oligo pools were ordered from Twist Bioscience (Carlsbad, Calif.) using the following designs: Target sequence:

Target3: (SEQ ID No. 19)
5' -CCAGTCAGTAATGTTACTGG,
and
Target7: (SEQ ID No. 20)
5' -AGCAGGACACTCCTGCCCA.

TABLE 2

Target Library Design:				
TARGET LIBRARY	PAM 5'	3' UMI	NUMBER OF PAMs FOR ANALYSIS	VARIANT/ DESIGN
PAM PANEL	TNNN	N	64	1
SPECIFICITY PANEL	TTTV	N	3	12

The PAM panel library was designed by adding TNNN randomized sequences as the 5'-end PAM for each target, then by adding a single bp N at the end of the target to be used as the unique molecular identifier in the sequencing analysis. The specificity panel was designed by introducing 2 bp tandem mismatches in the following positions in each target: 1st, 3rd, 7th, 8th, 9th, 11th, 13th, 14th, 15th, 17th, 18th, and 19th bp. Each target with 2 bp mismatches was used to add 5'-end TTTV PAM (IUPAC nomenclature: V=A, G, or C) and 3'-end 1 bp N as the UMI (unique molecule identifier) for sequencing analysis. The target library was cloned into a pUC19 backbone and prepared using the Midi-plus™ plasmid preparation kit (Qiagen). The target library pool was prepared at 10 ng/μL final concentration.

Example 5: In Vitro Transcription and Translation for Production of MAD70-Series Nucleases and gRNAs in a Single Well

A PURExpress® In Vitro Protein Synthesis Kit (NEB) was used to produce engineered MAD70-series variant proteins from the PCR-amplified MAD70-series variant library, and also to produce gRNAs for synthetic target Library of Target3 and Target7. In each well in a 96-well plate, the reagents in Table 3 were mixed to start the production of MAD7 variants and gRNA:

TABLE 3

Reagents			
REAGENTS		VOLUME (μl)	
1	SolA (NEB kit)	3.3	
2	SolB (NEB kit)	2.5	
3	gRNA mix (4 ng/μl stock)	0.8	
4	Murine RNase inhibitor (NEB)	0.2	

16

TABLE 3-continued

Reagents		
REAGENTS		VOLUME (μl)
5	Water	0.5
6	PCR amplified T7 MAD70-series variants	1.0

A master mix with all reagents was mixed on ice with the exception of the PCR-amplified T7-MAD70-series variants to cover enough 96-well plates for the assay. After 7.3 μL of the master mix was distributed in each well in 96 well plates, 1 μL of the PCR amplified MAD70-series variants under the control of T7 promoter was added. The 96-well plates were sealed and incubated for 4 hrs at 37° C. in a thermal cycler. The plates were kept at room temperature until the target pool was added to perform the target depletion reaction.

Example 6: Performing Target Depletion, PCR and NGS

After 4 hours incubation to allow production of the engineered MAD70-series variants and gRNAs, 4 μL of the target library pool (10 ng/μL) was added to the in vitro transcription/translation reaction mixture. After the target library was added, reaction mixtures were incubated overnight at 37° C. The target depletion reaction mixtures were diluted into PCR-grade water that contains RNase A and then boiled for 5 min at 95° C. The mixtures were then amplified and sequenced. The PCR conditions in Table 4 below were used:

TABLE 4

PCR Conditions		
STEP	TEMPERATURE	TIME
DENATURATION	98° C.	30 SEC
6 CYCLES	98° C.	10 SEC
	61° C.	30 SEC
	72° C.	10 SEC
	98° C.	10 SEC
22 CYCLES	98° C.	10 SEC
	72° C.	10 SEC
	72° C.	2 MINUTES
FINAL EXTENSION	72° C.	
HOLD	12° C.	

Example 7: Data Analysis

Table 5 is a table of amino acid substitutions made to the MAD7 nuclease amino acid sequence (SEQ ID No. 1) that result in MAD70-series variant nucleases with different PAM recognition sites as compared to the native MAD7 nuclease.

TABLE 5

MAD70-series Variants - Altered PAM Preference			
WT Residue	Mutation Detected	New PAMs, cut detected	SEQ ID No.
K535L	L	TGTN, TTCN	SEQ ID No. 2
K535S	S	TGTN, TTCN	SEQ ID No. 11
K535C	C	TGTN, TTCN	SEQ ID No. 12
K535R	R	TGTN, TTCN	SEQ ID No. 13
K535N	N	TGTN, TTCN	SEQ ID No. 14
K535G	G	TCTN as primary	SEQ ID No. 3

17

FIG. 1A is a heatmap for the MAD70-series variant nucleases with different PAM recognition sites. The K535R mutation disrupts the ability of the enzyme to recognize TCTN PAMs and enhances the ability of the enzyme to recognize PAMs containing a purine at the second position (TATN/TGTN). The K594 mutation ablates the recognition of the preferred TTTN PAMs while enhancing TCGN recognition.

Table 6 is a table of amino acid substitutions made to the MAD7 nuclease amino acid sequence (SEQ ID No. 1) resulting in MAD70-series variant nucleases with varied targeting fidelity as compared to the MAD7 reference nuclease.

TABLE 6

MAD70-series Variants - Varied Target Fidelity			
WT Residue	Mutation Detected	Pos_9 score for HF-MAD7 (wt > 0.3)	SEQ ID No.
R920G	G	0.0	SEQ ID No. 4
R924I	I	0.04	SEQ ID No. 5
K511L	L	0.03	SEQ ID No. 6
H283T	T	0.01	SEQ ID No. 7
R187K	K	0.0	SEQ ID No. 8
N589G	G	0.0	SEQ ID No. 9
K281A	A	0.04	SEQ ID No. 10
K281V	V	0.01	SEQ ID No. 15

FIG. 1B is the heatmap for the MAD70-series variant nucleases with varied fidelity as compared with wild-type MAD7 (SEQ ID No. 1). The bottom figure shows the PAM depletion panel for the same enzyme from the above figure. R187K and N589G showed better pos9 specificity but note from the bottom figure these MAD70-series nucleases showed reduced activity across all PAMs. As can be seen many of these mutations eliminate activity of the enzyme for targets that contain programmed 2 bp mismatches at the +9, +14, +15, and +17 positions relative to the PAM sequence indicating an improved targeting fidelity.

FIG. 2A is a PAM depletion vs specificity plot of the native MAD7 sequence (SEQ ID No. 1) sampled across multiple plates in a HT-screen. The PAM specificity is represented as the sum of the depletion scores observed for all PAMs tested (D_{PAM}) as calculated by Eqn 1:

$$PAM_{score} = \sum D_{PAM} \quad \text{eqn. 1:}$$

The relative nuclease specificity is calculated as the pos9_score as shown in eqn 2.

$$pos9_{score} = \frac{D_9}{D_{wt}} \quad \text{eqn. 2}$$

Where D_9 is the sum of the depletion scores for DNA target sequences containing a 2 bp mismatch at the PAM +9 position and D_{wt} is the sum of the depletion scores for DNA targets with perfect complementarity to the gRNAs used in this assay. This scoring methodology was chosen empirically based on the sensitivity of the targeting specificity to mutations in this register of the RNA-DNA interaction. Each point corresponds to an independent measurement from control digestion experiments run with the MAD7 nuclease (SEQ ID NO. 1). FIG. 2B is the plot for the screened 1108 single amino acid variants tested. Points in the lower two quadrants represent loss of function mutations which occurred in 433/1020 (43%) of the screened space. Data points in the upper left portion of the graph (>10 sum of

18

Pam_depletion, <0.3 pos_9_depletion/wt_depletion) represent variants that with high activity as judged by their summed PAM activity score and high altered RNA-guided enzyme fidelity relative to the wild-type MAD7 enzyme sequence (FIG. 2A).

Example 8: Combinatorial Mutation Library Construction, Screening and Data Analysis

Based on the results observed in Example 7, an additional mutant library was designed and screened for changes in PAM preference. The library was composed of mutations at both positions K535 and K594 (the residues are in relation to the MAD7 amino acid sequence in SEQ ID No. 1) substituting the original codon with NNK (IUPAC code for DNA: N=A, T, G, C; K=G, T) randomized codons. The library was constructed using a Q5 Site Directed Mutagenesis Kit (NEB) using manufacturers protocols with mutagenic forward 5'-TTCTNNKAACGCTATCATACTGATGC (SEQ ID No. 25) and reverse 5'-TACTCMNNGGACTTTGACCAACCGTC (SEQ ID No. 26) primers. The PCR reaction mix was transformed into 5-alpha chemically competent cells (NEB) and plated on LB agar plates with 100 µg/mL of carbenicillin. Colonies were picked and inoculated into 1 mL of LB medium (100 µg/mL of carbenicillin) in 96-well culture blocks and grown overnight in a shaking incubator at 37° C. Sample processing and screening was performed as described in examples 3, 4, 5 and 6.

FIG. 5 is a heatmap for a MAD70-series nuclease with novel PAM recognition sites identified from this library screening (SEQ ID No. 67). This mutant contains the combination of mutations K535R and N539S (SEQ ID No. 67) and results in more robust activity on PAMs with an A nucleotide at the second position of the NNNN PAM space, in particular TAAN, compared to the K535R mutation alone.

Example 9: Revised Target Library

A revised PAM panel library was designed by adding NNNN randomized sequences as the 5'-end PAM for each target, in order to evaluate activity on all 256 PAM sequences in the NNNN PAM space. Oligo pools were ordered from Twist Bioscience (Carlsbad, Calif.) using the following designs: Target3: 5'-CCAGTCAGTAATGT-TACTGG (SEQ ID No. 27), and Target7: 5'-AGCAGGACACTCCTGCCCA (SEQ ID No. 28). The target library was cloned into a pUC19 backbone and prepared using the Midi-plus™ plasmid preparation kit (Qiagen). The target library pool was prepared at 10 ng/µL final concentration.

TABLE 7

Revised Library				
Target Library	PAM 5'	3'UMI	Number of PAMs for analysis	Variant/Design
PAM panel (2 targets)	NNNN	none	256	1

Example 10: Mutagenic Library Construction, Screening and Data Analysis Using K535R/N539S Backbone

In order to further alter the PAM preference, a library of single amino acid mutations was generated using the

K535R/N539S mutant (SEQ ID No. 67) described in Example 8. Mutation sets were generated based on residue proximity to putative key regions of where the nuclease is predicted to interact with the PAM site, target, or gRNA, as well as targeting charged amino acids. The following amino acid residues were targeted for mutation (the residues are in relation to the MAD7 amino acid sequence in SEQ ID No. 1): 529, 530, 531, 532, 534, 536, 537, 538, 540, 541, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 599, 601, 650, 739, 740, 741, 742, 743. At each position, the original codon was substituted with NNK (IUPAC code for DNA: N=A, T, G, C; K=G, T) randomized codons. This library was screened for altered PAM preference as described in examples 3, 4, 5 and 6, using the target oligonucleotide library described in Example 9.

FIG. 6 represents activity heatmaps for wild-type MAD7 (SEQ ID No. 1) (FIG. 6A), the K535R/N539S mutant (SEQ ID No. 67) (FIG. 6B) used as the parent for this library, along with an additional MAD70-series nuclease with novel PAM recognition sites identified from this library (SEQ ID No. 68) (FIG. 6C). Data analysis was performed as described in Example 7, with heatmaps now representing activity on all 64 combinations of nucleotides in the NNNN PAM space. The new MAD70-series nuclease mutant (SEQ ID No. 68) contains the combination of mutations K535R/N539S/K594L/E730Q in relation to the wild-type MAD7 amino acid sequence in SEQ ID No. 1. It has novel activity on PAMs with a C nucleotide at the third position of the NNNN PAM space.

Example 11: Activity of MAD70-Series PAM Mutants in *Escherichia coli* Cells

In order to confirm activity of the MAD70-series mutants for genome editing systems in cells, activity was confirmed

using a phenotypic editing assay in *E. coli*. MAD70-series mutants were cloned into a EE0026 vector backbone. MAD70-series variants were amplified using reverse (5' GATGATTCTCTAGAGGTACTTAGAGATAGCGCTT-ATTCTGGATAAAGTC) (SEQ ID No. 29) and forward (5' CGATTCCGGAAAGGAGATATCTCAT-GAACAACGGCACAAATAATTTTCAG AA) (SEQ ID No. 30) primers and cloned into the linearized EE0026 Engine vector using the NEBuilder HF DNA assembly kit.

Editing cassettes were designed to introduce stop codons to disrupt the synthesis of full-length LacZ in *E. coli* as a result of editing. Each cassette was composed of a 20 base pair spacer to precisely target a region of lacZ gene in the *E. coli* genome adjacent to the indicated PAM sequence in the genome, and a 200 bp repair template for homologous recombination. DNA sequences and corresponding PAM targets for each cassette are provided in Table 8. Each cassette is cloned into the common cassette vector backbone p346BB (SEQ ID No. 87) using the NEBuilder HF DNA assembly kit. *E. coli* K-12 str MG1655 grown to mid-log phase in LB was made electrocompetent by washing three times with ice cold 10% glycerol. Engine vectors were transformed by electroporation, recovered in SOC for 1 hr at 30° C., then grown overnight on LB agar with Chloramphenicol (25 ug/mL) medium at 30° C. Overnight grown cells with MAD70-series variant engine vectors were grown to mid log phase in LB Chloramphenicol (25 ug/mL) and made competent with LB broth containing 10% (wt/vol) polyethylene glycol, 5% (vol/vol) dimethyl sulfoxide, and 50 mM Mg2+ at pH 6.5.

TABLE 8

Sequences of Editing cassettes and corresponding PAM targets				
Cassette name	Insert sequence	Target gene	PAM	SEQ ID No.
lacZ_127_TTTC_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGATAGATACCTGCCATAAAGA AACTGTCCATGTTGCCACTCGCTTTAATGATGATT TCAGCCGCGCTGACTGGAGGCTGAAGTTCAGAT GTGCGGCGAGTTGCGTGAACCTACGGTAACA TAATGATTATGGTAATGAGAGACCCAGGTCGCCA GCGGCACCGCGCTTTTCGGCGGTGAAATTATCGA TGAGCGTGGTGTATGCCGATCGCGTCACACTA ATCCAGAAAAGACCCGTCCG	lacZ	TTTC	31
lacZ_245_TTTC_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGATAGATCATGTTGCCACTCGC TTTAATCACCTGCCATAAAGAACTGTTACCCGT AGGTAGTCACGCAACTCGCCGACATCTGAACCT CAGCCTCCAGTACAGCGCGCTGAAATCATATTT CATTAAGTGGCTATTAGAGATAGCTGATTGTGT AGTCGGTTTATGCAGCAACGAGACGTCACGGAAA ATGCCGCTCATCCGCCATATCTGATCTTCATC CCAGAAAAGACCCGTCCG	lacZ	TTTC	32
lacZ_256_TTTG_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGATAGATTGATCGGTTTATG CAGCAACCGCTCGCGTGATGGTGCTGCGCTGG AGTGACGCGAGTTATCTGGAAGATCAGGATATGT GGCGGATGAGCGGCATTTCCGTGACGTCTCGTTG TAATGAAAACCGTAATGACAGATTAGCGATTTCC ATGTTGCCACTCGCTTTAATGATGATTTACGCCG GCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCA TCCCAGAAAAGACCCGTCCG	lacZ	TTTG	33

TABLE 8-continued

Sequences of Editing cassettes and corresponding PAM targets				
Cassette name	Insert sequence	Target gene	PAM	SEQ ID No.
lacZ_419_TTG_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGTAGATCCGTCTGAATTTGAC CTGAGCTCATCCGCCACATATCCTGATCTTCCAGA TAAGTCCGTCACCTCAGCGCAGACCATCACCG CGAGGCGGTTTCTCCGCGCGTAAAAATGCGCTT CATTAAAAATCTCATTACAGACCACTGTCTGGCC GTAACCGACCCAGCGCCGTTGCACCACAGATGA AACGCGAGTTAACGCCATCAAAAATAATTCGAT CCCAGAAAAGACCCGTCCG	lacZ	TTTG	34
lacZ_314_TATG_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGTAGATTGGCGGATGAGCGGC ATTTTCCAGTACAGCGCGGCTGAAATCATCATTA AAGCGAGTGGCAACATGGAAATCGCTGATTTGTG TAGTCGGTTTATGCAGCAACGAGACGTACCGGAA TCATTAGCTCATTCATTACACATTCTGATCTTCCA GATAACTGCCGTCACTCCAGCGCAGCACCATCAC CGCGAGGCGGTTTCTCCGCGCGTAAAAATGCA TCCCAGAAAAGACCCGTCCG	lacZ	TATG	35
lacZ_920_TATG_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGTAGATACCATGATTACGGAT TCACTCTATTACGCCAGCTGGCGAAAGGGGGATG TGCTGCAAGGCGATTAAAGTTGGGTAAACGCCAGGG TTTTCCCAGTCACGACGTTGTAAAAACGACGCCA GTCATTACGTAATTCATTACACTCATGTTCTCTGT GTGAAATGTTATCCGCTCACAATTCACACAACA TACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGA TCCCAGAAAAGACCCGTCCG	lacZ	TATG	36
lacZ_1712_AACG_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGTAGATCCATCAAAAATAATT CGCGTATTACGGTCAATCCGCCGTTTGTTCACAG GAGAAATCCGACGGGTTGTACTCGCTCACATTAA TGTTGATGAAAGCTGGCTACAGGAAGGCCAGACG TAATGAATTTTTTAATGAGTCAATTCGGCGTTTCA TCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGC CAGGACAGTCGTTTGCCGCTCTGAATTTGACCTATC CCAGAAAAGACCCGTCCG	lacZ	AACG	37
lacZ_466_AACG_stop	GTGTGTGATACGAAACGAAGCATTGGAGGCATTG GAATTTCTACTCTTGTAGATGGGATACTGACGAAA CGCCTAATGGCTTTGCTACCTGGAGAGACGCGC CCGCTGATCCTTTGCGAATACGCCACGCGATGG GTAACAGTCTTGCGGTTTTCGCTAAATACTGGCAG TAATGACGTGAGTAATGACGACTTCAGGGCGGCT TCGCTCTGGGACTGGGTGGATCGCTGATTAA ATATGATGAAAACGGCAACCCGTGGTTCGGCTTAC ATCCCAGAAAAGACCCGTCCG	lacZ	AACG	38

10 ng of editing cassette plasmid was added to 20 uL of chemically competent *E. coli* strain with an engine vector on ice. After 30 min, 250 uL of SOC was added and the cultures were incubated in a shaking incubator for 1 hr at 30° C. 30 uL of the resulting cultures were inoculated to 350 uL of LB Carbenicillin (100 ug/mL)/Chloramphenicol (25 ug/mL) and grown overnight in a shaking incubator at 30° C. 4 uL of the overnight cultures were inoculated to fresh 320 uL LB/Carbenicillin (100 ug/mL)/Chloramphenicol (25 ug/mL)/Arabinose (1% w/v) medium and incubated for 3 hrs in a shaking incubator at 30° C. Cultures were moved to a 42° C. shaking incubator to induce the production of RNP complex. After 5 hrs of induction at 42° C., cultures were moved back to the 30° C. shaking incubator and grown overnight. Overnight grown edited strains were spotted on a MacConkey Agar Plates (Teknova) and grown overnight at 37° C. without any antibiotics. Cultures with native LacZ can ferment the lactose in the medium, produces acid that

lowers the pH that makes the red color in the colony. Cultures with edited disrupted LacZ can't ferment the lactose and the colonies grow colorless. A summary of editing phenotypes observed for MAD70-series PAM mutants is shown in FIG. 7. Darker spots indicate intact lacZ and lighter spots are cells with lacZ that are edited and thus are non-functional, indicating gene editing activity on the PAMs listed at top.

Example 12: Phenotypic Assay to Measure Genome Editing for MAD7-Derived Mutants in *Saccharomyces cerevisiae* Cells

To assess the genome editing activity of RNA-guided nucleases in *S. cerevisiae*, a two micron plasmid was constructed for the sequential introduction of DNA containing an editing cassette with SNR52 promoter-driven crRNA and a CYC1 promoter-driven nuclease protein (see FIG. 8). The editing cassette comprises the crRNA to guide the nuclease

23

to cut at a specific DNA sequence, a short linker, and a repair template containing the mutation of interest flanked by regions of homology to the genome. The screening plasmid (FIG. 8) was linearized by the *Stu*I restriction endonuclease, and the editing cassette was introduced downstream of the SNR52p promoter by isothermal assembly. The editing cassettes inserted into the *Stu*I-linearized plasmid for the introduction of a premature stop codon into the *can1* gene, organized by the PAM of the corresponding spacer, are shown in Table 9. The nuclease proteins were amplified by polymerase chain reaction with oligonucleotide primers to introduce an SV40 nuclear localization sequence at the N-terminus consisting of the DNA sequence "ATGGCACC-CAAGAAGAAGAGGAAGGTGTTA" (SEQ ID No. 39) corresponding to a protein sequence of "MAPKKRKVL" (SEQ ID No. 40)." The resulting amplified DNA fragment (400 ng, purified) was then co-transformed along with a *Psi*I-linearized screening plasmid (250 ng) that already contained an editing cassette to assemble the complete

24

editing plasmid by in vivo gap repair. Cells containing a repaired plasmid were selected for in yeast peptone-dextrose (YPD) containing 200 mg/L Geneticin for 3 days at 30 degrees C. in a humidified shaking incubator. The resulting saturated culture was diluted 1:80 into synthetic complete yeast media lacking arginine and containing 50 mg/L of canavanine and grown overnight at 30 degrees C. in a humidified shaking incubator. Because knockout of the *Can1* protein allows yeast to grow in the presence of the otherwise toxic analog canavanine, the relative OD600 of the overnight cultures is proportional to the rate of genome mutation induced by the transformed nuclease protein. The MAD70-series variants described in Examples 7 and 8 with altered PAM preference were evaluated in the assay system using the editing cassettes shown in Table 9, targeting various PAMs. The results of this analysis are shown in FIG. 9, where the mutant containing mutations K535R, K539S in reference to the wild-type MAD7 sequence shows substantially higher editing activity on TATV PAMs.

TABLE 9

Editing cassettes targeting yeast <i>can1</i> gene to introduce loss of function mutations		
Cassette name	PAM Editing Cassette Sequence	SEQ ID No.
Can1_S30stop	TTTA GGCCCCAAATTCTAATTTCTACTGTTGTAGATAC GACGTTGAAGCTTCACAATTTTACGCCGACAT AGAGGAGAAGCATATGTACAATGAGCCGGTCAC AACCCTCGAGACACGACGTTGAAGCTTAACAAA CACACCACAGACGTGGGTCAATACCATTGAAAG ATGAGAAAAGTAACAATATACGCGCTCCTGCC	41
Can1_K42stop	TTTA GGCCCCAAATTCTAATTTCTACTGTTGTAGATCT TTTCTCATCTTTCAATGGTTTTGTATCCTCGCCA TTTACTCTCGTCGGAAAGAGCGCAATGGATAC AATCCCACTTTTCTCATCTTACAATGGTATTG ACCCACGTCGTGGTGTGTTTGTGAAGCTTCAAC GTCGTCAATATACGCGCTCCTGCC	42
Can1_N60stop	TTTC GGCCCCAAATTCTAATTTCTACTGTTGTAGATCC GACGAGAGTAAATGGCGATTTTTCATACCAT TGAAAGATGAGAAAAGTAAAGAATTGTATCCAT TGCGCTCGTTCCCGACGAGATATAAGGCGAGG ATACGTTCTCTATGGAGGATGGCATAGGTGATG AAGATGAAGGAGAAGCAATATACGCGCTCCTGCCC	43
Can1_T115stop	TTTA GGCCCCAAATTCTAATTTCTACTGTTGTAGATTC CACACCTCTGACCAACGCTTTTTATTGGTATGAT TGCCCTTGGTGGTACTATGGTACAGGTCTTTTC ATTGGATTATCCACACCTCTGTAAACGCCGGC CCAGTGGCGCTCTTATATCATATTTATTTATGG GTTCTTTGGCATCAATATACGCGCTCCTGCC	44
Can1_Q158stop	TTTC GGCCCCAAATTCTAATTTCTACTGTTGTAGATAC AGTTTTCTCACAAGATTTTTTCTGTACACGCA GTCCTTGGGTGAAATGGCTACATTCATCCCTGTT ACATCCTCGTTCACAGTTTTCTCATAAAGATTCC TTTCTCCAGCATTTGGTGGCGCAATGGTTACAT GTATTGGTTTTCAATATACGCGCTCCTGCC	45
Can1_I214stop	TTTG GGCCCCAAATTCTAATTTCTACTGTTGTAGATGG TAATTATACAATAATGATTTTTCATTCAATTTT GGACGTACAAAGTTCCTACTGGCGGCATGGATTA GTATTGGGAAGTAATTATCACATAAATGAACT TGTTCCTGTCAAATATTACGGTGAAATTCGAGTT CTGGGTCGCAATATACGCGCTCCTGCC	46
Can1_G72stop	TCTA GGCCCCAAATTCTAATTTCTACTGTTGTAGATTG GAGGATGGCATAGGTGATTTTAAATTGTATCCA TTGCGCTCTTTCCCGACGAGAGTAAATGGCGAG	47

TABLE 9-continued

Editing cassettes targeting yeast can1 gene to introduce loss of function mutations		
Cassette name	PAM Editing Cassette Sequence	SEQ ID No.
	GATACGTTTCTCCATGGAGGATGGCATATAAGAT GAAGATGAAGGAGAAGTACAGAACGCTGAAGT GAAGAGAGAGCTTAACAATATACGCGCTCCTGCCC	
Can1_Q80stop	TCTC GGCCCCAAATTCTAATTTCTACTGTTGTAGATTT CACTTCAGCGTTCTGTACTTTTCCAAATAGTACC ACCAAGGGCAATCATACCAATATGTCTTTGCTT AAGTCCCCCTTCACTTCAGCGTTTATACTTCT CCTTCATCTTCATCACCTATGCCATCCTCCATAG AGAACGTATCAATATACGCGCTCCTGCCC	48
Can1_E142stop	TGTC GGCCCCAAATTCTAATTTCTACTGTTGTAGATAC GCAGTCCTTGGGTGAAATTTTCCAGTGGGCGC TCTTATATCATATTTATTTATGGGTTCTTTGGCAT ATTGCGTCACGCAGTCCTTGGGTAAATGGCTA CATTTCATCCCTGTTACATCCTCTTTCACAGTTTTC TCACAAAGATCAATATACGCGCTCCTGCCC	49
Can1_S152stop	TGTG GGCCCCAAATTCTAATTTCTACTGTTGTAGATAG AAAACGTGAAAGAGGATTTTTTAACCAATACA TGTAACCATTTGGCCGCACCAATGCTGGAGAAA GGAATCTCCCTGAGAAAAGTGAATTAGGATG TAACAGGGATGAATGTAGCCATTTACCCAAAGG ACTGCGTGACAGCAATATACGCGCTCCTGCCC	50
Can1_V20stop	TATG GGCCCCAAATTCTAATTTCTACTGTTGTAGATTA CAATGAGCCGGTCACAACTTTGGCATAGCAA TGACAAATTCAAAAGAAGACGCCGACATAGAG GAGAAGCACGGGTACAATGAGCCGTAAACAAC CCTCTTTCACGACGTTGAAGCTTCAACAACACA CCACAGACGTGGGTCAACAATATACGCGCTCCT GCCC	51
Can1_N116stop	TATC GGCCCCAAATTCTAATTTCTACTGTTGTAGATCA CACCTCTGACCAACGCCGTTTTGTATGATTGCC CTTGGTGGTACTATTGGTACAGGTCTTTTCATTG GTTTAAGTACACCTCTGACCTAAGCCGCCAG TGGGCGCTCTTATATCATATTTATTTATGGGTTTC TTTGGCATATTCCAATATACGCGCTCCTGCCC	52

Example 13: Testing of MAD7 Variant Proteins for Enhanced Genome Editing in *S. cerevisiae*

To screen libraries of MAD7 enzyme variants with one or more mutations for increased genome editing activity in *S. cerevisiae*, six different editing cassettes (all targeting the TTTV PAM class) (first six entries in Table 9 (SEQ ID Nos. 41-46)) were inserted into the StuI-linearized two micron screening plasmid (again see FIG. 8) as described in Example 12. MAD7 protein variant coding sequences as described in Example 2 were amplified by polymerase chain reaction with oligonucleotide primers to introduce an SV40 nuclear localization sequence at the N-terminus consisting of the DNA sequence “ATGGCACCCAAGAAGAAGAG-GAAGGTGTTA” (SEQ ID No. 39) corresponding to a protein sequence of “MAPKKKRRKVL (SEQ ID No. 40).” The resulting amplified DNA fragment (5 uL of crude PCR mixture) was then co-transformed along with a PsiI-linearized screening plasmid (150 ng total, a pool of all 6 editing cassettes) that already contains an editing cassette to assemble the complete editing plasmid by in vivo gap repair. Cells containing a repaired plasmid were selected for in yeast peptone-dextrose (YPD) containing 200 mg/L Geneticin for 3 days at 30° C. in a humidified shaking incubator. The resulting saturated culture was diluted into synthetic complete yeast media lacking arginine and containing 50

mg/L of canavanine and grown overnight at 30° C. in a humidified shaking incubator. Because knockout of the Can1 protein allows yeast to grow in the presence of the otherwise toxic analog canavanine, the relative OD600 of the overnight cultures is proportional to the rate of genome mutation induced by the transformed nuclease protein. The relative genome editing activity levels of each variant are plotted in FIG. 10. Rescreening of the variants in quadruplicate in the original assay confirmed the enhanced genome editing activity of several MAD70-series variants, as shown in FIG. 11. Sequences are provided in SEQ ID Nos. 69 (K95L), 70 (V201I/K278T), 71 (K511D), 72 (N589H), 73 (L597V), 74 (K712V), 75 (E743I), 76 (K786S), 77 (K853R), and 78 (R1113F).

Example 14: Generation of Combinatorial MAD7 Variant Libraries and Screening for Enhanced Editing in *S. cerevisiae*

Based on the identified single mutations that enhance the genome editing activity of MAD7 in *S. cerevisiae*, combinatorial libraries were prepared. The N589H MAD70-series variant sequence (SEQ ID No. 72) was used as a backbone and 4 to 5 additional mutations were introduced using oligonucleotide primers and the Quick-Change Lightning Multi-Site Mutagenesis kit (Agilent) according to manufac-

27

turer instructions. These variants were screened for genome editing activity in *S. cerevisiae* as described in Example 12 as depicted in FIG. 12. The variants that showed enhanced activity in the primary screen were rescreened in quadruplicate and the results of the secondary screening are depicted in FIG. 13. Sequences are provided in SEQ ID Nos. 79 (S124T/K511I/N589H/K712V/K853R/H946W), 80 (S124T/K511I/N589H/K786S/H946K/R113F), 81 (K511H/N589H/K853R/K1021L/D118E/DE11833), 82 (K95L/S124T/K511I/N589H), 83 (S124T/K511I/N589H/K721I/K786S/K1021V), 84 (S124T/K511H/N589H/K853R/H946K/K1054Y), 85 (K95T/S124T/N589H/K853R/K1052Q), and 86 (S124T/K511T/N589H/K712L/H946T/K1052Q/K1054N).

Example 15: Activity of MAD70-Series PAM Mutants in Mammalian Cells

Wild-type MAD7 and MAD70-series variants with altered PAM preference were cloned downstream of a CAG promoter for strong expression in mammalian cells. The vector sequence used for expression is provided in SEQ ID No. 89. Guide RNAs (gRNAs) targeting various PAMs were cloned downstream of a U6 promoter in the backbone vector sequence provided in SEQ ID No. 90. Transfections in HEK293T cells were performed using 100 ng of total DNA (gRNA/MAD70-series variant plasmid) and Lipofectamine 3000 transfection reagent. The transfection mix was added to cells that had been cultured in 96 well plates 24 hrs prior to transfection. To measure indels, T7E1 assay was performed. Cells were lysed by the addition of a buffer containing proteinase K and incubation at 56° C. for 30 minutes. Proteinase K was inactivated by heating the reaction to 95° C. for 10 minutes. Following lysis, 10 uL PCR reactions were performed using genomic template from lysed cells and 2xQ5 PCR mastermix (NEB) to amplify amplicons containing the target sites that were edited. Following PCR, the PCR fragments were heated to 95° C. for 5 minutes and slowly cooled to room temperature. Then, T7 endonuclease I (NEB) was added to the PCR reaction and incubated for 1 hour at 37° C. The reaction was then resolved on 2.5% agarose gel and imaged using GelDoc (BioRad). The band intensities on the gel were quantified to calculate indels introduced by MAD7. The results are shown in FIG. 14. The MAD70-series mutant containing mutations K535R/N539S (SEQ ID No. 67) in reference to the wild-type MAD7 sequence shows substantially higher editing activity on TATC PAM while the K535R/N539S/K594L/E730Q (SEQ ID No. 68) mutant in relation to wild-type MAD7 shows higher editing on ATCC and TTCC PAMs.

28

Table 10: Sequences of Spacers and the PAM Sequences that were Targeted in the PPIB Locus

Target #	PAM	Spacer Sequence	SEQ ID No.
1	CTTC	cctccccctagcaacgccccctt	53
2	CATA	ggattttttaccgtcaccaaaa	54
3	AATA	tggtctctattctctctcccat	55
4	ATCG	gctgaactctgcaggtcagtt	56
5	ATCC	tcagggttagcttctgtacct	57
6	AATC	agattcagaaccacttctcta	58
7	TATC	ctgtagtccaaggagggtata	59
8	TATA	gataagcatgttttccaagaa	60
9	AACG	cccccttaagaagctaagtt	61
10	AACC	acttctctaaaaatattggctc	62
11	TTTT	tcagattcagaaccacttctc	63
12	TTTT	tatggctctattctctctccc	64
13	ATTC	tctctcccactcctcaggtag	65
14	TTCC	tcagggtgtattttgacctacg	66

While this invention is satisfied by embodiments in many different forms, as described in detail in connection with preferred embodiments of the invention, it is understood that the present disclosure is to be considered as exemplary of the principles of the invention and is not intended to limit the invention to the specific embodiments illustrated and described herein. Numerous variations may be made by persons skilled in the art without departure from the spirit of the invention. The scope of the invention will be measured by the appended claims and their equivalents. The abstract and the title are not to be construed as limiting the scope of the present invention, as their purpose is to enable the appropriate authorities, as well as the general public, to quickly determine the general nature of the invention. In the claims that follow, unless the term “means” is used, none of the features or elements recited therein should be construed as means-plus-function limitations pursuant to 35 U.S.C. § 112, ¶6.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 90

<210> SEQ ID NO 1

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Eubacterium rectale

<400> SEQUENCE: 1

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln

-continued

20						25						30				
Gln	Phe	Ile	Val	Lys	Asn	Gly	Ile	Ile	Lys	Glu	Asp	Glu	Leu	Arg	Gly	
		35					40					45				
Glu	Asn	Arg	Gln	Ile	Leu	Lys	Asp	Ile	Met	Asp	Asp	Tyr	Tyr	Arg	Gly	
	50					55					60					
Phe	Ile	Ser	Glu	Thr	Leu	Ser	Ser	Ile	Asp	Asp	Ile	Asp	Trp	Thr	Ser	
65					70					75					80	
Leu	Phe	Glu	Lys	Met	Glu	Ile	Gln	Leu	Lys	Asn	Gly	Asp	Asn	Lys	Asp	
			85						90					95		
Thr	Leu	Ile	Lys	Glu	Gln	Thr	Glu	Tyr	Arg	Lys	Ala	Ile	His	Lys	Lys	
			100					105					110			
Phe	Ala	Asn	Asp	Asp	Arg	Phe	Lys	Asn	Met	Phe	Ser	Ala	Lys	Leu	Ile	
		115					120					125				
Ser	Asp	Ile	Leu	Pro	Glu	Phe	Val	Ile	His	Asn	Asn	Asn	Tyr	Ser	Ala	
	130					135					140					
Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe	
145					150					155					160	
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser	
			165						170					175		
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn	
		180						185					190			
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys	
	195					200					205					
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp	
	210					215					220					
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr	
	225				230					235					240	
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys	
			245						250					255		
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu	
		260						265					270			
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys	
		275					280					285				
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu	
	290					295					300					
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys	
	305				310					315					320	
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr	
			325						330					335		
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser	
		340						345					350			
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile	
		355					360					365				
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys	
	370					375					380					
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile	
	385				390					395					400	
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys	
			405					410						415		
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu	
		420						425					430			
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu	
	435						440					445				

-continued

Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala	
450	455 460
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp	
465	470 475 480
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro	
	485 490 495
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln Lys Pro	
	500 505 510
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala	
	515 520 525
Asp Gly Trp Ser Lys Ser Lys Glu Tyr Ser Asn Asn Ala Ile Ile Leu	
	530 535 540
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys	
	545 550 555 560
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp	
	565 570 575
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro Asn Lys Met Ile	
	580 585 590
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro	
	595 600 605
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser	
	610 615 620
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe	
	625 630 635 640
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp	
	645 650 655
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu	
	660 665 670
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys	
	675 680 685
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile	
	690 695 700
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His	
	705 710 715 720
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile	
	725 730 735
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser	
	740 745 750
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg	
	755 760 765
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val	
	770 775 780
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe	
	785 790 795 800
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys	
	805 810 815
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr	
	820 825 830
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn	
	835 840 845
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr	
	850 855 860

-continued

Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	
865					870					875					880	
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val	
				885					890					895		
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys	
			900					905					910			
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys	
		915					920					925				
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val	
	930					935					940					
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala	
945					950					955					960	
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu	
			965					970						975		
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn	
			980					985					990			
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu	
		995					1000					1005				
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val		
	1010					1015					1020					
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr		
	1025					1030					1035					
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe		
	1040					1045					1050					
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe		
	1055					1060					1065					
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr		
	1070					1075					1080					
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys		
	1085					1090					1095					
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg		
	1100					1105					1110					
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile		
	1115					1120					1125					
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp		
	1130					1135					1140					
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile		
	1145					1150					1155					
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg		
	1160					1165					1170					
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile		
	1175					1180					1185					
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys		
	1190					1195					1200					
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr		
	1205					1210					1215					
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu		
	1220					1225					1230					
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile		
	1235					1240					1245					
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu		
	1250					1255					1260					

-continued

```

<210> SEQ ID NO 2
<211> LENGTH: 1263
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MAD70 VARIANT K535L

<400> SEQUENCE: 2

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1              5              10              15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20              25              30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35              40              45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50              55              60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65              70              75              80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85              90              95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
100              105              110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
115              120              125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
130              135              140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
145              150              155              160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
165              170              175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
180              185              190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
195              200              205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
210              215              220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
225              230              235              240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
245              250              255

Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
260              265              270

Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys
275              280              285

Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu
290              295              300

Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys
305              310              315              320

His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr
325              330              335

Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser
340              345              350

Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile
355              360              365

His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys

```

-continued

370	375	380
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile 385 390 395 400		
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys 405 410 415		
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu 420 425 430		
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu 435 440 445		
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala 450 455 460		
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp 465 470 475 480		
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro 485 490 495		
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln Lys Pro 500 505 510		
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala 515 520 525		
Asp Gly Trp Ser Lys Ser Leu Glu Tyr Ser Asn Asn Ala Ile Ile Leu 530 535 540		
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys 545 550 555 560		
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp 565 570 575		
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro Asn Lys Met Ile 580 585 590		
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro 595 600 605		
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser 610 615 620		
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe 625 630 635 640		
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp 645 650 655		
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu 660 665 670		
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys 675 680 685		
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile 690 695 700		
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His 705 710 715 720		
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile 725 730 735		
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser 740 745 750		
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg 755 760 765		
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val 770 775 780		
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe 785 790 795 800		

-continued

Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys
 805 810 815
 Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr
 820 825 830
 Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn
 835 840 845
 Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr
 850 855 860
 Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu
 865 870 875 880
 Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val
 885 890 895
 Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys
 900 905 910
 Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925
 Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940
 Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960
 Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975
 Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990
 Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005
 Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020
 Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035
 Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050
 Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065
 Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080
 Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095
 Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110
 Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125
 Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140
 Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155
 Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170
 Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185
 Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200

-continued

Ala Gly	Asp Ala Leu Pro Lys	Asp Ala Asp Ala Asn	Gly Ala Tyr
1205	1210	1215	
Cys Ile	Ala Leu Lys Gly Leu	Tyr Glu Ile Lys Gln	Ile Thr Glu
1220	1225	1230	
Asn Trp	Lys Glu Asp Gly Lys	Phe Ser Arg Asp Lys	Leu Lys Ile
1235	1240	1245	
Ser Asn	Lys Asp Trp Phe Asp	Phe Ile Gln Asn Lys	Arg Tyr Leu
1250	1255	1260	

<210> SEQ ID NO 3
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K594G

<400> SEQUENCE: 3

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser	
1 5 10 15	
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln	
20 25 30	
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly	
35 40 45	
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly	
50 55 60	
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser	
65 70 75 80	
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp	
85 90 95	
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys	
100 105 110	
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile	
115 120 125	
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala	
130 135 140	
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe	
145 150 155 160	
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser	
165 170 175	
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn	
180 185 190	
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys	
195 200 205	
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp	
210 215 220	
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr	
225 230 235 240	
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys	
245 250 255	
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu	
260 265 270	
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys	
275 280 285	
Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu	
290 295 300	

-continued

Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys	305	310	315	320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr	325	330	335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser	340	345	350	
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile	355	360	365	
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys	370	375	380	
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile	385	390	395	400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys	405	410	415	
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu	420	425	430	
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu	435	440	445	
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala	450	455	460	
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp	465	470	475	480
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro	485	490	495	
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro	500	505	510	
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	515	520	525	
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	530	535	540	
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	545	550	555	560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp	565	570	575	
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile	580	585	590	
Pro	Gly	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro	595	600	605	
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser	610	615	620	
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe	625	630	635	640
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp	645	650	655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu	660	665	670	
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	675	680	685	
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	690	695	700	
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	705	710	715	720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	725	730	735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	740	745	750	
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	755	760	765	

Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
770						775				780					
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe
785						790				795		800			
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys
		805						810				815			
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr
		820						825				830			
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn
835						840						845			
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr
850						855				860					
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu
865						870				875		880			
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val
		885						890				895			
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys
		900						905				910			
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys
915						920						925			
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val
930						935						940			
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala
945						950				955		960			
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu
		965						970				975			
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn
		980						985				990			
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu
995						1000						1005			
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val	
1010						1015						1020			
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr	
1025						1030						1035			
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe	
1040						1045						1050			
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe	
1055						1060						1065			
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr	
1070						1075						1080			
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys	
1085						1090						1095			
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg	
1100						1105						1110			
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile	
1115						1120						1125			
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp	
1130						1135						1140			
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile	
1145															

-continued

1175	1180	1185
Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys		
1190	1195	1200
Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr		
1205	1210	1215
Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu		
1220	1225	1230
Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile		
1235	1240	1245
Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu		
1250	1255	1260

<210> SEQ ID NO 4
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT R920G

<400> SEQUENCE: 4

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser	
1 5 10 15	
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln	
20 25 30	
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly	
35 40 45	
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly	
50 55 60	
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser	
65 70 75 80	
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp	
85 90 95	
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys	
100 105 110	
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile	
115 120 125	
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala	
130 135 140	
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe	
145 150 155 160	
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser	
165 170 175	
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn	
180 185 190	
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys	
195 200 205	
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp	
210 215 220	
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr	
225 230 235 240	
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys	
245 250 255	
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu	
260 265 270	
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys	

-continued

275	280	285
Ile Ala Asp Thr Ser Tyr 290	Glu Val Pro Tyr Lys 295	Phe Glu Ser Asp Glu 300
Glu Val Tyr Gln Ser 305	Val Asn Gly Phe Leu Asp 310	Asn Ile Ser Ser Lys 315
His Ile Val Glu Arg 325	Leu Arg Lys Ile Gly Asp 330	Asn Tyr Asn Gly Tyr 335
Asn Leu Asp Lys Ile Tyr 340	Ile Val Ser Lys Phe Tyr 345	Glu Ser Val Ser 350
Gln Lys Thr Tyr Arg Asp 355	Trp Glu Thr Ile Asn Thr 360	Ala Leu Glu Ile 365
His Tyr Asn Asn Ile Leu 370	Pro Gly Asn Gly Lys 375	Ser Lys Ala Asp Lys 380
Val Lys Lys Ala Val Lys 385	Asn Asp Leu Gln Lys 390	Ser Ile Thr Glu Ile 395
Asn Glu Leu Val Ser Asn 405	Tyr Lys Leu Cys Ser Asp 410	Asp Asn Ile Lys 415
Ala Glu Thr Tyr Ile His 420	Glu Ile Ser His Ile Leu 425	Asn Asn Phe Glu 430
Ala Gln Glu Leu Lys Tyr 435	Asn Pro Glu Ile His Leu 440	Val Glu Ser Glu 445
Leu Lys Ala Ser Glu Leu 450	Lys Asn Val Leu Asp 455	Val Ile Met Asn Ala 460
Phe His Trp Cys Ser Val 465	Phe Met Thr Glu Glu 470	Leu Val Asp Lys Asp 475
Asn Asn Phe Tyr Ala Glu 485	Leu Glu Glu Ile Tyr Asp 490	Glu Ile Tyr Pro 495
Val Ile Ser Leu Tyr Asn 500	Leu Val Arg Asn Tyr 505	Val Thr Gln Lys Pro 510
Tyr Ser Thr Lys Lys Ile 515	Lys Leu Asn Phe Gly 520	Ile Pro Thr Leu Ala 525
Asp Gly Trp Ser Lys Ser 530	Lys Glu Tyr Ser Asn 535	Asn Ala Ile Ile Leu 540
Met Arg Asp Asn Leu Tyr 545	Tyr Leu Gly Ile Phe 550	Asn Ala Lys Asn Lys 555
Pro Asp Lys Lys Ile Ile 565	Glu Gly Asn Thr Ser 570	Glu Asn Lys Gly Asp 575
Tyr Lys Lys Met Ile Tyr 580	Asn Leu Leu Pro Gly 585	Pro Asn Lys Met Ile 590
Pro Lys Val Phe Leu Ser 595	Ser Lys Thr Gly Val 600	Glu Thr Tyr Lys Pro 605
Ser Ala Tyr Ile Leu Glu 610	Gly Tyr Lys Gln Asn 615	Lys His Ile Lys Ser 620
Ser Lys Asp Phe Asp Ile 625	Thr Phe Cys His Asp 630	Leu Ile Asp Tyr Phe 635
Lys Asn Cys Ile Ala Ile 645	His Pro Glu Trp Lys 650	Asn Phe Gly Phe Asp 655
Phe Ser Asp Thr Ser Thr 660	Tyr Glu Asp Ile Ser 665	Gly Phe Tyr Arg Glu 670
Val Glu Leu Gln Gly Tyr 675	Lys Ile Asp Trp Thr 680	Tyr Ile Ser Glu Lys 685
Asp Ile Asp Leu Leu Gln 690	Glu Lys Gly Gln Leu 695	Tyr Leu Phe Gln Ile 700

-continued

Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
705					710					715					720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile
			725						730					735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser
			740					745					750		
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg
		755					760					765			
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
		770				775					780				
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe
		785			790					795					800
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys
			805						810					815	
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr
			820					825					830		
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn
		835					840					845			
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr
	850					855					860				
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu
	865				870					875					880
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val
			885						890					895	
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys
		900						905					910		
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Gly	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys
		915					920					925			
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val
	930					935					940				
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala
	945				950					955					960
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu
			965					970						975	
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn
		980						985					990		
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu
	995						1000						1005		
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val	
	1010					1015						1020			
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr	
	1025					1030					1035				
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe	
	1040					1045						1050			
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe	
	1055					1060					1065				
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr	
	1070					1075						1080			
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys	
	1085					1090						1095			
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg	
	1100					1105						1110			

-continued

Phe Val	Asn Gly	Arg Phe	Ser	Asn Glu	Ser Asp	Thr	Ile Asp	Ile	
1115			1120			1125			
Thr Lys	Asp Met	Glu Lys	Thr	Leu Glu	Met Thr	Asp	Ile Asn	Trp	
1130			1135			1140			
Arg Asp	Gly His	Asp Leu	Arg	Gln Asp	Ile Ile	Asp	Tyr Glu	Ile	
1145			1150			1155			
Val Gln	His Ile	Phe Glu	Ile	Phe Arg	Leu Thr	Val	Gln Met	Arg	
1160			1165			1170			
Asn Ser	Leu Ser	Glu Leu	Glu	Asp Arg	Asp Tyr	Asp	Arg Leu	Ile	
1175			1180			1185			
Ser Pro	Val Leu	Asn Glu	Asn	Asn Ile	Phe Tyr	Asp	Ser Ala	Lys	
1190			1195			1200			
Ala Gly	Asp Ala	Leu Pro	Lys	Asp Ala	Asp Ala	Asn	Gly Ala	Tyr	
1205			1210			1215			
Cys Ile	Ala Leu	Lys Gly	Leu	Tyr Glu	Ile Lys	Gln	Ile Thr	Glu	
1220			1225			1230			
Asn Trp	Lys Glu	Asp Gly	Lys	Phe Ser	Arg Asp	Lys	Leu Lys	Ile	
1235			1240			1245			
Ser Asn	Lys Asp	Trp Phe	Asp	Phe Ile	Gln Asn	Lys	Arg Tyr	Leu	
1250			1255			1260			

<210> SEQ ID NO 5
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT R924I

<400> SEQUENCE: 5

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser	
1 5 10 15	
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln	
20 25 30	
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly	
35 40 45	
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly	
50 55 60	
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser	
65 70 75 80	
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp	
85 90 95	
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys	
100 105 110	
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile	
115 120 125	
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala	
130 135 140	
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe	
145 150 155 160	
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser	
165 170 175	
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn	
180 185 190	
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys	
195 200 205	

-continued

Ser 210	Leu	Ser	Asn	Asp	Asp	Ile 215	Asn	Lys	Ile	Ser	Gly 220	Asp	Met	Lys	Asp
Ser 225	Leu	Lys	Glu	Met	Ser 230	Leu	Glu	Glu	Ile	Tyr 235	Ser	Tyr	Glu	Lys	Tyr 240
Gly	Glu	Phe	Ile	Thr 245	Gln	Glu	Gly	Ile	Ser 250	Phe	Tyr	Asn	Asp	Ile	Cys 255
Gly	Lys	Val	Asn 260	Ser	Phe	Met	Asn 265	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu 270
Asn	Lys	Asn 275	Leu	Tyr	Lys	Leu	Gln 280	Lys	Leu	His	Lys	Gln 285	Ile	Leu	Cys
Ile	Ala	Asp	Thr	Ser	Tyr	Glu 295	Val	Pro	Tyr	Lys	Phe 300	Glu	Ser	Asp	Glu
Glu 305	Val	Tyr	Gln	Ser	Val 310	Asn	Gly	Phe	Leu	Asp	Asn 315	Ile	Ser	Ser	Lys 320
His	Ile	Val	Glu	Arg 325	Leu	Arg	Lys	Ile	Gly 330	Asp	Asn	Tyr	Asn	Gly	Tyr 335
Asn	Leu	Asp	Lys 340	Ile	Tyr	Ile	Val	Ser 345	Lys	Phe	Tyr	Glu	Ser	Val	Ser
Gln	Lys	Thr 355	Tyr	Arg	Asp	Trp	Glu 360	Thr	Ile	Asn	Thr	Ala 365	Leu	Glu	Ile
His 370	Tyr	Asn	Asn	Ile	Leu	Pro 375	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Asp	Lys
Val 385	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys	Ser 395	Ile	Thr	Glu	Ile 400
Asn	Glu	Leu	Val	Ser 405	Asn	Tyr	Lys	Leu	Cys	Ser 410	Asp	Asp	Asn	Ile	Lys 415
Ala	Glu	Thr	Tyr	Ile 420	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn	Asn	Phe	Glu 430
Ala	Gln	Glu	Leu	Lys	Tyr	Asn 440	Pro	Glu	Ile	His	Leu	Val 445	Glu	Ser	Glu
Leu 450	Lys	Ala	Ser	Glu	Leu 455	Lys	Asn	Val	Leu	Asp	Val 460	Ile	Met	Asn	Ala
Phe 465	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu	Leu 475	Val	Asp	Lys	Asp 480
Asn	Asn	Phe	Tyr	Ala 485	Glu	Leu	Glu	Glu	Ile 490	Tyr	Asp	Glu	Ile	Tyr	Pro 495
Val	Ile	Ser	Leu	Tyr 500	Asn	Leu	Val	Arg 505	Asn	Tyr	Val	Thr	Gln	Lys	Pro 510
Tyr	Ser	Thr	Lys	Lys	Ile	Lys 520	Leu	Asn	Phe	Gly	Ile	Pro 525	Thr	Leu	Ala
Asp 530	Gly	Trp	Ser	Lys	Ser 535	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu 540
Met 545	Arg	Asp	Asn	Leu	Tyr 550	Tyr	Leu	Gly	Ile	Phe	Asn 555	Ala	Lys	Asn	Lys 560
Pro	Asp	Lys	Lys	Ile 565	Ile	Glu	Gly	Asn	Thr 570	Ser	Glu	Asn	Lys	Gly	Asp 575
Tyr	Lys	Lys	Met	Ile 580	Tyr	Asn	Leu	Leu	Pro 585	Gly	Pro	Asn	Lys	Met	Ile 590
Pro	Lys	Val	Phe	Leu	Ser	Ser 600	Lys	Thr	Gly	Val	Glu	Thr 605	Tyr	Lys	Pro
Ser 610	Ala	Tyr	Ile	Leu	Glu	Gly 615	Tyr	Lys	Gln	Asn	Lys 620	His	Ile	Lys	Ser
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe

-continued

625	630	635	640
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp			
	645	650	655
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu			
	660	665	670
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys			
	675	680	685
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile			
	690	695	700
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His			
	705	710	715
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile			
	725	730	735
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser			
	740	745	750
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg			
	755	760	765
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val			
	770	775	780
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe			
	785	790	795
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys			
	805	810	815
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr			
	820	825	830
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn			
	835	840	845
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr			
	850	855	860
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu			
	865	870	875
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val			
	885	890	895
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys			
	900	905	910
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Ile Lys Glu Trp Lys			
	915	920	925
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val			
	930	935	940
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala			
	945	950	955
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu			
	965	970	975
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn			
	980	985	990
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu			
	995	1000	1005
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val			
	1010	1015	1020
Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr			
	1025	1030	1035
Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe			
	1040	1045	1050

-continued

Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065
 Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080
 Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095
 Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110
 Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125
 Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140
 Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155
 Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170
 Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185
 Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200
 Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215
 Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230
 Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245
 Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 6
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K511L

<400> SEQUENCE: 6

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15
 Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30
 Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35 40 45
 Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50 55 60
 Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65 70 75 80
 Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85 90 95
 Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
 100 105 110
 Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
 115 120 125
 Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
 130 135 140

-continued

Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe	
145					150					155					160	
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser	
			165						170					175		
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn	
			180					185					190			
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys	
	195					200					205					
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp	
	210				215						220					
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr	
	225				230					235					240	
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys	
			245					250						255		
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu	
		260						265					270			
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys	
		275				280						285				
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu	
	290					295					300					
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys	
	305				310					315					320	
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr	
			325						330					335		
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser	
		340						345					350			
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile	
		355				360						365				
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys	
	370				375						380					
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile	
	385				390					395					400	
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys	
		405						410						415		
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu	
		420						425					430			
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu	
	435					440						445				
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala	
	450				455					460						
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp	
	465				470					475					480	
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro	
		485						490						495		
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Leu	Pro	
		500						505					510			
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	
		515					520					525				
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	
	530					535						540				
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	
	545				550					555					560	

Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp	
				565					570					575		
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile	
				580					585					590		
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro	
				595					600					605		
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser	
				610					615					620		
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe	
				625					630					635		
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp	
				645					650					655		
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu	
				660					665					670		
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	
				675					680					685		
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	
				690					695					700		
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	
				705					710					715		
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	
				725					730					735		
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	
				740					745					750		
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	
				755					760					765		
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	
				770					775					780		
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	
				785					790					795		
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	
				805					810					815		
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	
				820					825					830		
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	
				835					840					845		
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr	
				850					855					860		
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	
				865					870					875		
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val	
				885					890					895		
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys	
				900					905					910		
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys	
				915					920					925		
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val	
				930					935					940		
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala	
				945					950					955		
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val		

-continued

980					985					990					
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu
	995						1000					1005			
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val	
	1010					1015					1020				
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr	
	1025					1030					1035				
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe	
	1040					1045					1050				
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe	
	1055					1060					1065				
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr	
	1070					1075					1080				
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys	
	1085					1090					1095				
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg	
	1100					1105					1110				
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile	
	1115					1120					1125				
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp	
	1130					1135					1140				
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile	
	1145					1150					1155				
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg	
	1160					1165					1170				
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile	
	1175					1180					1185				
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys	
	1190					1195					1200				
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr	
	1205					1210					1215				
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu	
	1220					1225					1230				
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile	
	1235					1240					1245				
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu	
	1250					1255					1260				

<210> SEQ ID NO 7

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT H283T

<400> SEQUENCE: 7

Met	Asn	Asn	Gly	Thr	Asn	Asn	Phe	Gln	Asn	Phe	Ile	Gly	Ile	Ser	Ser
1				5				10						15	

Leu	Gln	Lys	Thr	Leu	Arg	Asn	Ala	Leu	Ile	Pro	Thr	Glu	Thr	Thr	Gln
	20					25						30			

Gln	Phe	Ile	Val	Lys	Asn	Gly	Ile	Ile	Lys	Glu	Asp	Glu	Leu	Arg	Gly
	35					40						45			

Glu	Asn	Arg	Gln	Ile	Leu	Lys	Asp	Ile	Met	Asp	Asp	Tyr	Tyr	Arg	Gly
	50				55					60					

Phe	Ile	Ser	Glu	Thr	Leu	Ser	Ser	Ile	Asp	Asp	Ile	Asp	Trp	Thr	Ser
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

-continued

65	70	75	80
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp	85	90	95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys	100	105	110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile	115	120	125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala	130	135	140
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe	145	150	155
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser	165	170	175
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn	180	185	190
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys	195	200	205
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp	210	215	220
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr	225	230	235
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys	245	250	255
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu	260	265	270
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu Thr Lys Gln Ile Leu Cys	275	280	285
Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu	290	295	300
Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys	305	310	315
His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr	325	330	335
Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser	340	345	350
Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile	355	360	365
His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys	370	375	380
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile	385	390	395
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys	405	410	415
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu	420	425	430
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu	435	440	445
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala	450	455	460
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp	465	470	475
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro	485	490	495

Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro
			500					505					510		
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
		515					520					525			
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
	530					535					540				
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
545					550					555					560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
				565					570					575	
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile
			580					585					590		
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
		595					600					605			
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
	610					615					620				
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
625					630					635					640
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
			645						650					655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
			660					665					670		
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
		675					680					685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
	690					695					700				
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
705					710					715					720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile
			725						730					735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser
			740					745				750			
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg
		755					760					765			
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
	770					775					780				
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe
785					790					795					800
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys
			805						810					815	
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr
			820					825					830		
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn
			835				840					845			
Phe	Lys	Ala													

-continued

Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925
 Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940
 Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960
 Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975
 Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990
 Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005
 Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020
 Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035
 Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050
 Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065
 Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080
 Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095
 Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110
 Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125
 Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140
 Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155
 Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170
 Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185
 Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200
 Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215
 Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230
 Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245
 Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 8

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT R187K

<400> SEQUENCE: 8

-continued

Met	Asn	Asn	Gly	Thr	Asn	Asn	Phe	Gln	Asn	Phe	Ile	Gly	Ile	Ser	Ser	1	5	10	15
Leu	Gln	Lys	Thr	Leu	Arg	Asn	Ala	Leu	Ile	Pro	Thr	Glu	Thr	Thr	Gln	20	25	30	
Gln	Phe	Ile	Val	Lys	Asn	Gly	Ile	Ile	Lys	Glu	Asp	Glu	Leu	Arg	Gly	35	40	45	
Glu	Asn	Arg	Gln	Ile	Leu	Lys	Asp	Ile	Met	Asp	Asp	Tyr	Tyr	Arg	Gly	50	55	60	
Phe	Ile	Ser	Glu	Thr	Leu	Ser	Ser	Ile	Asp	Asp	Ile	Asp	Trp	Thr	Ser	65	70	75	80
Leu	Phe	Glu	Lys	Met	Glu	Ile	Gln	Leu	Lys	Asn	Gly	Asp	Asn	Lys	Asp	85	90	95	
Thr	Leu	Ile	Lys	Glu	Gln	Thr	Glu	Tyr	Arg	Lys	Ala	Ile	His	Lys	Lys	100	105	110	
Phe	Ala	Asn	Asp	Asp	Arg	Phe	Lys	Asn	Met	Phe	Ser	Ala	Lys	Leu	Ile	115	120	125	
Ser	Asp	Ile	Leu	Pro	Glu	Phe	Val	Ile	His	Asn	Asn	Asn	Tyr	Ser	Ala	130	135	140	
Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe	145	150	155	160
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser	165	170	175	
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Lys	Ile	Val	Asn	Asp	Asn	180	185	190	
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys	195	200	205	
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp	210	215	220	
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr	225	230	235	240
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys	245	250	255	
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu	260	265	270	
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys	275	280	285	
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu	290	295	300	
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys	305	310	315	320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr	325	330	335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser	340	345	350	
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile	355	360	365	
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys	370	375	380	
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile	385	390	395	400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys	405	410	415	
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu				

-continued

420							425					430				
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu	
		435					440					445				
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala	
		450					455				460					
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp	
		465					470				475				480	
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro	
				485					490					495		
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro	
			500					505					510			
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	
			515				520					525				
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	
		530					535				540					
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	
		545					550				555				560	
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp	
				565					570					575		
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile	
			580					585					590			
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro	
			595				600					605				
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser	
		610					615				620					
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe	
		625					630				635				640	
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp	
				645					650					655		
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu	
			660					665					670			
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	
			675					680					685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	
		690					695				700					
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	
					710					715					720	
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	
				725					730					735		
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	
			740					745					750			
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	
			755				760					765				
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	
			770				775				780					
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	
					790					795					800	
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	
				805					810					815		
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	
			820					825					830			
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	
			835				840					845				

-continued

Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr
850						855					860				
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu
865					870					875				880	
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val
			885						890					895	
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys
		900						905					910		
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys
		915						920				925			
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val
	930					935					940				
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala
945					950					955					960
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu
			965						970						975
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn
			980						985					990	
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu
		995						1000					1005		
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val	
	1010					1015						1020			
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr	
	1025					1030						1035			
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe	
	1040					1045						1050			
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe	
	1055					1060						1065			
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr	
	1070					1075						1080			
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys	
	1085					1090						1095			
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg	
	1100					1105						1110			
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile	
	1115					1120						1125			
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp	
	1130					1135						1140			
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile	
	1145					1150						1155			
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg	
	1160					1165						1170			
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile	
	1175					1180						1185			
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys	
	1190					1195						1200			
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr	
	1205					1210						1215			
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu	
	1220					1225						1230			
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile	
	1235					1240						1245			

-continued

Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu
1250						1255						1260		

<210> SEQ ID NO 9
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT N589G

<400> SEQUENCE: 9

Met	Asn	Asn	Gly	Thr	Asn	Asn	Phe	Gln	Asn	Phe	Ile	Gly	Ile	Ser	Ser
1				5					10					15	
Leu	Gln	Lys	Thr	Leu	Arg	Asn	Ala	Leu	Ile	Pro	Thr	Glu	Thr	Thr	Gln
			20					25					30		
Gln	Phe	Ile	Val	Lys	Asn	Gly	Ile	Ile	Lys	Glu	Asp	Glu	Leu	Arg	Gly
			35				40					45			
Glu	Asn	Arg	Gln	Ile	Leu	Lys	Asp	Ile	Met	Asp	Asp	Tyr	Tyr	Arg	Gly
	50					55				60					
Phe	Ile	Ser	Glu	Thr	Leu	Ser	Ser	Ile	Asp	Asp	Ile	Asp	Trp	Thr	Ser
65					70					75				80	
Leu	Phe	Glu	Lys	Met	Glu	Ile	Gln	Leu	Lys	Asn	Gly	Asp	Asn	Lys	Asp
			85					90						95	
Thr	Leu	Ile	Lys	Glu	Gln	Thr	Glu	Tyr	Arg	Lys	Ala	Ile	His	Lys	Lys
			100					105					110		
Phe	Ala	Asn	Asp	Asp	Arg	Phe	Lys	Asn	Met	Phe	Ser	Ala	Lys	Leu	Ile
		115					120					125			
Ser	Asp	Ile	Leu	Pro	Glu	Phe	Val	Ile	His	Asn	Asn	Asn	Tyr	Ser	Ala
	130						135					140			
Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe
145					150					155					160
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser
			165					170						175	
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn
		180						185					190		
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys
		195					200				205				
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp
	210					215					220				
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr
225				230						235				240	
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys
			245					250						255	
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu
		260						265					270		
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys
		275					280					285			
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu
	290						295				300				
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys
305					310					315					320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
		325						330						335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
		340						345					350		

Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
355						360						365			
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
370						375				380					
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
385				390						395				400	
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
				405				410						415	
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
		420						425				430			
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
		435				440						445			
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
450						455				460					
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp
465				470						475				480	
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro
		485						490						495	
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro
		500						505				510			
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
		515				520						525			
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
530						535				540					
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
545				550						555				560	
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
		565						570						575	
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Gly	Lys	Met	Ile
		580						585				590			
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
		595				600						605			
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
610						615				620					
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
625				630						635				640	
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
		645						650						655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
		660						665				670			
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
		675				680						685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
690						695				700					
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
705				710						715				720	
Thr	Met</														

-continued

770	775	780
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe		
785	790	795 800
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys		
	805	810 815
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr		
	820	825 830
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn		
	835	840 845
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr		
	850	855 860
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu		
	865	870 875 880
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val		
	885	890 895
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys		
	900	905 910
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys		
	915	920 925
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val		
	930	935 940
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala		
	945	950 955 960
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu		
	965	970 975
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn		
	980	985 990
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu		
	995	1000 1005
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val		
	1010	1015 1020
Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr		
	1025	1030 1035
Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe		
	1040	1045 1050
Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe		
	1055	1060 1065
Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr		
	1070	1075 1080
Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys		
	1085	1090 1095
Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg		
	1100	1105 1110
Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile		
	1115	1120 1125
Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp		
	1130	1135 1140
Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile		
	1145	1150 1155
Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg		
	1160	1165 1170
Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile		
	1175	1180 1185

-continued

Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200

Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215

Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230

Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245

Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 10
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K281A

<400> SEQUENCE: 10

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35 40 45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50 55 60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65 70 75 80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85 90 95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
 100 105 110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
 115 120 125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
 130 135 140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
 145 150 155 160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
 165 170 175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
 180 185 190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
 195 200 205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
 210 215 220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
 225 230 235 240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
 245 250 255

Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
 260 265 270

Asn Lys Asn Leu Tyr Lys Leu Gln Ala Leu His Lys Gln Ile Leu Cys
 275 280 285

-continued

Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu
290						295					300				
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys
305					310					315					320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
				325					330					335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
			340						345					350	
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
			355				360					365			
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
					370		375				380				
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
385					390					395					400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
				405					410					415	
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
				420				425						430	
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
				435			440					445			
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
				450			455				460				
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp
465					470					475					480
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro
				485					490					495	
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro
			500					505					510		
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
			515				520					525			
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
			530			535					540				
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
545					550					555					560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
				565				570						575	
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile
			580					585					590		
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
			595				600					605			
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
			610			615					620				
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
625					630					635					640
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
				645					650					655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
				660				665					670		
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
			675				680					685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
			690			695					700				

-continued

Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	705	710	715	720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	725	730	735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	740	745	750	
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	755	760	765	
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	770	775	780	
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	785	790	795	800
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	805	810	815	
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	820	825	830	
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	835	840	845	
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr	850	855	860	
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	865	870	875	880
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val	885	890	895	
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys	900	905	910	
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys	915	920	925	
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val	930	935	940	
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala	945	950	955	960
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu	965	970	975	
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn	980	985	990	
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu	995	1000	1005	
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val		1010	1015	1020	
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr		1025	1030	1035	
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe		1040	1045	1050	
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe		1055	1060	1065	
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr		1070	1075	1080	
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys		1085	1090	1095	
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg		1100	1105	1110	
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile					

-continued

1115	1120	1125
Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp		
1130	1135	1140
Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile		
1145	1150	1155
Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg		
1160	1165	1170
Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile		
1175	1180	1185
Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys		
1190	1195	1200
Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr		
1205	1210	1215
Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu		
1220	1225	1230
Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile		
1235	1240	1245
Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu		
1250	1255	1260

<210> SEQ ID NO 11

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT K535S

<400> SEQUENCE: 11

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser		
1	5	10
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln		
20	25	30
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly		
35	40	45
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly		
50	55	60
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser		
65	70	75
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp		
85	90	95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys		
100	105	110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile		
115	120	125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala		
130	135	140
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe		
145	150	155
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser		
165	170	175
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn		
180	185	190
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys		
195	200	205
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp		

-continued

210	215	220
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr		
225	230	235 240
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys		
	245	250 255
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu		
	260	265 270
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys		
	275	280 285
Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu		
	290	295 300
Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys		
	305	310 315 320
His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr		
	325	330 335
Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser		
	340	345 350
Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile		
	355	360 365
His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys		
	370	375 380
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile		
	385	390 395 400
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys		
	405	410 415
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu		
	420	425 430
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu		
	435	440 445
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala		
	450	455 460
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp		
	465	470 475 480
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro		
	485	490 495
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln Lys Pro		
	500	505 510
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala		
	515	520 525
Asp Gly Trp Ser Lys Ser Ser Glu Tyr Ser Asn Asn Ala Ile Ile Leu		
	530	535 540
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys		
	545	550 555 560
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp		
	565	570 575
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro Asn Lys Met Ile		
	580	585 590
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro		
	595	600 605
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser		
	610	615 620
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe		
	625	630 635 640

-continued

Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp
 645 650 655

Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu
 660 665 670

Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys
 675 680 685

Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile
 690 695 700

Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His
 705 710 715 720

Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile
 725 730 735

Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser
 740 745 750

Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg
 755 760 765

Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val
 770 775 780

Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe
 785 790 795 800

Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys
 805 810 815

Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr
 820 825 830

Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn
 835 840 845

Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr
 850 855 860

Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu
 865 870 875 880

Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val
 885 890 895

Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys
 900 905 910

Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925

Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940

Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960

Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975

Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990

Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005

Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020

Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035

Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050

-continued

Lys Asp 1055	Leu Thr Val Asp 1060	Lys Arg Glu Phe Ile 1065	Lys Lys Phe
Asp Ser 1070	Ile Arg Tyr Asp Ser 1075	Glu Lys Asn Leu Phe 1080	Cys Phe Thr
Phe Asp 1085	Tyr Asn Asn Phe Ile 1090	Thr Gln Asn Thr Val 1095	Met Ser Lys
Ser Ser 1100	Trp Ser Val Tyr Thr 1105	Tyr Gly Val Arg Ile 1110	Lys Arg Arg
Phe Val 1115	Asn Gly Arg Phe Ser 1120	Asn Glu Ser Asp Thr 1125	Ile Asp Ile
Thr Lys 1130	Asp Met Glu Lys Thr 1135	Leu Glu Met Thr Asp 1140	Ile Asn Trp
Arg Asp 1145	Gly His Asp Leu Arg 1150	Gln Asp Ile Ile Asp 1155	Tyr Glu Ile
Val Gln 1160	His Ile Phe Glu Ile 1165	Phe Arg Leu Thr Val 1170	Gln Met Arg
Asn Ser 1175	Leu Ser Glu Leu Glu 1180	Asp Arg Asp Tyr Asp 1185	Arg Leu Ile
Ser Pro 1190	Val Leu Asn Glu Asn 1195	Asn Ile Phe Tyr Asp 1200	Ser Ala Lys
Ala Gly 1205	Asp Ala Leu Pro Lys 1210	Asp Ala Asp Ala Asn 1215	Gly Ala Tyr
Cys Ile 1220	Ala Leu Lys Gly Leu 1225	Tyr Glu Ile Lys Gln 1230	Ile Thr Glu
Asn Trp 1235	Lys Glu Asp Gly Lys 1240	Phe Ser Arg Asp Lys 1245	Leu Lys Ile
Ser Asn 1250	Lys Asp Trp Phe Asp 1255	Phe Ile Gln Asn Lys 1260	Arg Tyr Leu

<210> SEQ ID NO 12
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K535C

<400> SEQUENCE: 12

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser 1 5 10 15
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln 20 25 30
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly 35 40 45
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly 50 55 60
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser 65 70 75 80
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp 85 90 95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys 100 105 110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile 115 120 125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala 130 135 140

-continued

Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe	145	150	155	160
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser	165	170	175	
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn	180	185	190	
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys	195	200	205	
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp	210	215	220	
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr	225	230	235	240
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys	245	250	255	
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu	260	265	270	
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys	275	280	285	
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu	290	295	300	
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys	305	310	315	320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr	325	330	335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser	340	345	350	
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile	355	360	365	
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys	370	375	380	
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile	385	390	395	400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys	405	410	415	
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu	420	425	430	
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu	435	440	445	
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala	450	455	460	
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp	465	470	475	480
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro	485	490	495	
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro	500	505	510	
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	515	520	525	
Asp	Gly	Trp	Ser	Lys	Ser	Cys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	530	535	540	
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	545	550	555	560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp				

-continued

565								570					575				
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile		
			580					585					590				
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro		
		595					600					605					
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser		
	610					615					620						
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe		
	625				630					635					640		
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp		
			645						650					655			
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu		
			660					665					670				
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys		
		675					680					685					
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile		
	690					695					700						
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His		
	705				710					715					720		
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile		
			725						730					735			
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser		
			740					745					750				
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg		
		755					760					765					
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val		
	770					775					780						
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe		
	785				790					795					800		
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys		
			805						810					815			
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr		
			820					825					830				
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn		
		835					840					845					
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr		
	850					855					860						
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu		
	865				870					875					880		
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val		
			885						890					895			
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys		
		900						905					910				
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys		
		915					920					925					
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val		
	930					935					940						
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala		
	945				950					955					960		
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu		
			965						970					975			
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn		
			980					985					990				

-continued

Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005
 Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020
 Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035
 Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050
 Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065
 Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080
 Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095
 Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110
 Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125
 Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140
 Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155
 Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170
 Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185
 Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200
 Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215
 Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230
 Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245
 Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 13
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K535R

<400> SEQUENCE: 13

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15
 Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30
 Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35 40 45
 Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50 55 60
 Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65 70 75 80

Leu	Phe	Glu	Lys	Met	Glu	Ile	Gln	Leu	Lys	Asn	Gly	Asp	Asn	Lys	Asp
85									90					95	
Thr	Leu	Ile	Lys	Glu	Gln	Thr	Glu	Tyr	Arg	Lys	Ala	Ile	His	Lys	Lys
100								105					110		
Phe	Ala	Asn	Asp	Asp	Arg	Phe	Lys	Asn	Met	Phe	Ser	Ala	Lys	Leu	Ile
115							120					125			
Ser	Asp	Ile	Leu	Pro	Glu	Phe	Val	Ile	His	Asn	Asn	Asn	Tyr	Ser	Ala
130						135					140				
Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe
145					150					155					160
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser
165									170					175	
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn
180								185					190		
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys
195							200					205			
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp
210						215					220				
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr
225					230					235					240
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys
245								250						255	
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu
260								265					270		
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys
275							280					285			
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu
290						295					300				
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys
305					310					315					320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
325									330					335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
340								345					350		
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
355							360					365			
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
370						375					380				
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
385					390					395					400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
405								410					415		
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
420								425					430		
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
435							440					445			
Leu	Lys	Ala	Ser	Glu	Leu	Lys									

Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro	
			500					505					510			
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	
		515					520					525				
Asp	Gly	Trp	Ser	Lys	Ser	Arg	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	
	530					535					540					
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	
545					550					555					560	
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp	
				565					570					575		
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile	
			580					585					590			
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro	
		595					600					605				
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser	
					615						620					
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe	
625					630					635					640	
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp	
				645					650					655		
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu	
			660					665					670			
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	
							680					685				
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	
	690					695					700					
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	
705					710					715					720	
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	
				725					730					735		
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	
			740					745					750			
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	
			755				760					765				
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	
	770					775					780					
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	
785					790					795					800	
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	
			805						810					815		
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	
			820					825					830			
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr										

-continued

915				920				925							
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val
930						935					940				
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala
945					950					955					960
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu
					965					970					975
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn
					980					985					990
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu
					995					1000					1005
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val	
	1010						1015								1020
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr	
	1025						1030								1035
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe	
	1040						1045								1050
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe	
	1055						1060								1065
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr	
	1070						1075								1080
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys	
	1085						1090								1095
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg	
	1100						1105								1110
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile	
	1115						1120								1125
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp	
	1130						1135								1140
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile	
	1145						1150								1155
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg	
	1160						1165								1170
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile	
	1175						1180								1185
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys	
	1190						1195								1200
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr	
	1205						1210								1215
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu	
	1220						1225								1230
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile	
	1235						1240								1245
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu	
	1250						1255								1260

<210> SEQ ID NO 14

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT K535N

<400> SEQUENCE: 14

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser

-continued

1	5	10	15
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln	20	25	30
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly	35	40	45
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly	50	55	60
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser	65	70	75
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp	85	90	95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys	100	105	110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile	115	120	125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala	130	135	140
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe	145	150	155
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser	165	170	175
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn	180	185	190
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys	195	200	205
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp	210	215	220
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr	225	230	235
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys	245	250	255
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu	260	265	270
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys	275	280	285
Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu	290	295	300
Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys	305	310	315
His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr	325	330	335
Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser	340	345	350
Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile	355	360	365
His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys	370	375	380
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile	385	390	395
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys	405	410	415
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu	420	425	430

Ala 435	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
Leu 450	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
Phe 465	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
Asp	Gly	Trp	Ser	Lys	Ser	Asn	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
Met 545	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
Ser 625	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
Tyr 705	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
Arg 785	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn

Phe 850	Lys	Ala	Asn	Lys	Thr	Gly 855	Phe	Ile	Asn	Asp	Arg 860	Ile	Leu	Gln	Tyr
Ile 865	Ala	Lys	Glu	Lys	Asp 870	Leu	His	Val	Ile	Gly 875	Ile	Asp	Arg	Gly	Glu 880
Arg	Asn	Leu	Ile	Tyr 885	Val	Ser	Val	Ile	Asp 890	Thr	Cys	Gly	Asn	Ile	Val 895
Glu	Gln	Lys	Ser	Phe 900	Asn	Ile	Val	Asn 905	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys 910
Leu	Lys	Gln	Gln	Glu	Gly 915	Ala	Arg	Gln	Ile	Ala	Arg 925	Lys	Glu	Trp	Lys 930
Glu	Ile	Gly	Lys	Ile	Lys 935	Glu	Ile	Lys	Glu	Gly	Tyr 940	Leu	Ser	Leu	Val 945
Ile	His	Glu	Ile	Ser 950	Lys	Met	Val	Ile	Lys	Tyr	Asn 955	Ala	Ile	Ile	Ala 960
Met	Glu	Asp	Leu	Ser 965	Tyr	Gly	Phe	Lys	Lys 970	Gly	Arg	Phe	Lys	Val	Glu 975
Arg	Gln	Val	Tyr 980	Gln	Lys	Phe	Glu	Thr	Met 985	Leu	Ile	Asn	Lys	Leu	Asn 990
Tyr	Leu	Val	Phe 995	Lys	Asp	Ile	Ser	Ile	Thr 1000	Glu	Asn	Gly	Gly	Leu	Leu 1005
Lys	Gly 1010	Tyr	Gln	Leu	Thr 1015	Tyr	Ile	Pro	Asp	Lys	Leu 1020	Lys	Asn	Val	
Gly	His 1025	Gln	Cys	Gly	Cys 1030	Ile	Phe	Tyr	Val	Pro	Ala 1035	Ala	Tyr	Thr	
Ser	Lys 1040	Ile	Asp	Pro	Thr 1045	Thr	Gly	Phe	Val	Asn	Ile 1050	Phe	Lys	Phe	
Lys	Asp 1055	Leu	Thr	Val	Asp 1060	Ala	Lys	Arg	Glu	Phe	Ile 1065	Lys	Lys	Phe	
Asp	Ser 1070	Ile	Arg	Tyr	Asp 1075	Ser	Glu	Lys	Asn	Leu	Phe 1080	Cys	Phe	Thr	
Phe	Asp 1085	Tyr	Asn	Asn	Phe 1090	Ile	Thr	Gln	Asn	Thr	Val 1095	Met	Ser	Lys	
Ser	Ser 1100	Trp	Ser	Val	Tyr 1105	Thr	Tyr	Gly	Val	Arg	Ile 1110	Lys	Arg	Arg	
Phe	Val 1115	Asn	Gly	Arg	Phe 1120	Ser	Asn	Glu	Ser	Asp	Thr 1125	Ile	Asp	Ile	
Thr	Lys 1130	Asp	Met	Glu	Lys 1135	Thr	Leu	Glu	Met	Thr	Asp 1140	Ile	Asn	Trp	
Arg	Asp 1145	Gly	His	Asp	Leu 1150	Arg	Gln	Asp	Ile	Ile	Asp 1155	Tyr	Glu	Ile	
Val	Gln 1160	His	Ile	Phe	Glu 1165	Ile	Phe	Arg	Leu	Thr	Val 1170	Gln	Met	Arg	
Asn	Ser 1175	Leu	Ser	Glu	Leu 1180	Glu	Asp	Arg	Asp	Tyr	Asp 1185	Arg	Leu	Ile	
Ser	Pro 1190	Val	Leu	Asn	Glu 1195	Asn	Asn	Ile	Phe	Tyr	Asp 1200	Ser	Ala	Lys	
Ala	Gly 1205	Asp	Ala	Leu	Pro 1210	Lys	Asp	Ala	Asp	Ala	Asn 1215	Gly	Ala	Tyr	
Cys	Ile 1220	Ala	Leu	Lys	Gly 1225	Leu	Tyr	Glu	Ile	Lys	Gln 1230	Ile	Thr	Glu	
Asn	Trp 1235	Lys	Glu	Asp	Gly 1240	Lys	Phe	Ser	Arg	Asp	Lys 1245	Leu	Lys	Ile	
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu	

-continued

355					360					365					
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
370						375					380				
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
385					390					395					400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
				405					410					415	
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
			420					425					430		
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
			435				440					445			
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
450						455					460				
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp
465					470					475					480
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro
				485					490					495	
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro
			500					505					510		
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
			515				520					525			
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
530						535					540				
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
545					550					555					560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
				565					570					575	
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile
			580					585					590		
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
		595					600					605			
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
610						615					620				
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
625					630					635					640
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
				645					650					655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
			660					665					670		
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
		675					680					685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
690						695					700				
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
705					710					715					720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile
				725					730					735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser
			740					745					750		
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg
			755					760				765			
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
770						775					780				

-continued

Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe			
785	790	795	800
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys			
	805	810	815
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr			
	820	825	830
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn			
	835	840	845
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr			
	850	855	860
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu			
	865	870	875
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val			
	885	890	895
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys			
	900	905	910
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys			
	915	920	925
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val			
	930	935	940
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala			
	945	950	955
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu			
	965	970	975
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn			
	980	985	990
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu			
	995	1000	1005
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val			
	1010	1015	1020
Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr			
	1025	1030	1035
Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe			
	1040	1045	1050
Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe			
	1055	1060	1065
Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr			
	1070	1075	1080
Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys			
	1085	1090	1095
Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg			
	1100	1105	1110
Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile			
	1115	1120	1125
Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp			
	1130	1135	1140
Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile			
	1145	1150	1155
Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg			
	1160	1165	1170
Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile			
	1175	1180	1185

-continued

Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys
1190						1195					1200			
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr
1205						1210					1215			
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu
1220						1225					1230			
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile
1235						1240					1245			
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu
1250						1255					1260			

<210> SEQ ID NO 16
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 16

ttgggtaacg ccagggtttt

20

<210> SEQ ID NO 17
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 17

tgtgtggaat tgtgagcgga

20

<210> SEQ ID NO 18
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: GRNA SCAFFOLD SEQUENCE

<400> SEQUENCE: 18

ggaatttcta ctctttaga t

21

<210> SEQ ID NO 19
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: TARGET SEQUENCE

<400> SEQUENCE: 19

ccagtcagta atgttactgg

20

<210> SEQ ID NO 20
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: TARGET SEQUENCE

<400> SEQUENCE: 20

agcaggacac tcctgccccca

20

<210> SEQ ID NO 21
 <211> LENGTH: 97
 <212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: GRNA CONSTRUCT

<400> SEQUENCE: 21

cgaattaata cgactcacta tagggggaat ttctactctt gtageaggac actcctgccc 60
 cataactagc ataacccttc tctaacggag gggtttg 97

<210> SEQ ID NO 22
 <211> LENGTH: 34
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: GRNA CONSTRUCT

<400> SEQUENCE: 22

Arg Ile Leu Val Val Ser Leu Leu Pro Phe Lys Leu Glu Gln Leu Tyr
 1 5 10 15

Cys Ser Val Gly Ala Gly Tyr Ser Ala Tyr Gly Arg Glu Leu Arg Leu
 20 25 30

Pro Lys

<210> SEQ ID NO 23
 <211> LENGTH: 106
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: GRNA CONSTRUCT

<400> SEQUENCE: 23

gaattaatac gactcactat agggggaatt tctactcttg tagatccagt ccagtcagta 60
 atgttactgg taactagcat aaccctcttc taaacggagg ggttga 106

<210> SEQ ID NO 24
 <211> LENGTH: 35
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: GRNA CONSTRUCTS

<400> SEQUENCE: 24

Arg Ile Leu Val Val Ser Leu Leu Pro Phe Lys Leu Glu Gln Leu Asp
 1 5 10 15

Leu Ser Tyr His Leu Gln Tyr Ser Ala Tyr Gly Arg Glu Leu Arg Leu
 20 25 30

Pro Lys Phe
 35

<210> SEQ ID NO 25
 <211> LENGTH: 26
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PRIMER
 <220> FEATURE:
 <221> NAME/KEY: N
 <222> LOCATION: (5)..(5)
 <223> OTHER INFORMATION: N = ANY NUCLEOTIDE
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (5)..(7)
 <223> OTHER INFORMATION: n is a, c, g, or t
 <220> FEATURE:
 <221> NAME/KEY: N
 <222> LOCATION: (6)..(6)

-continued

```

<223> OTHER INFORMATION: N = ANY NUCLEOTIDE
<220> FEATURE:
<221> NAME/KEY: N
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: N = G OR T

```

```

<400> SEQUENCE: 25

```

```

ttctnnnaac gctatcatatc tgatgc

```

26

```

<210> SEQ ID NO 26
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PRIMER
<220> FEATURE:
<221> NAME/KEY: N
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: N = A OR C
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (6)..(8)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: N
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: N = ANY NUCLEOTIDE
<220> FEATURE:
<221> NAME/KEY: N
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: N = ANY NUCLEOTIDE

```

```

<400> SEQUENCE: 26

```

```

tactcnnngg actttgacca accgctc

```

26

```

<210> SEQ ID NO 27
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TARGET SEQUENCE

```

```

<400> SEQUENCE: 27

```

```

ccagtcagta atgttactgg

```

20

```

<210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TARGET SEQUENCE

```

```

<400> SEQUENCE: 28

```

```

agcaggacac tcctgccccca

```

20

```

<210> SEQ ID NO 29
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PRIMER

```

```

<400> SEQUENCE: 29

```

```

gatgatttct ctagaggtac ttagagatag cgcttattct ggataaagtc

```

50

```

<210> SEQ ID NO 30
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

```

-continued

<220> FEATURE:
 <223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 30

cgattccgga aaggagatat ctcatgaaca acggcacaaa taattttcag aa 52

<210> SEQ ID NO 31
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 31

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agataccctg 60

ccataaagaa actgtccatg ttgccactcg ctttaatgat gatttcagcc gcgctgtact 120

ggaggctgaa gttcagatgt gcggcgagtt gcgtgactac ctacgggtaa cataatgatt 180

atggtaatga gagacccagg tcgccagcgg caccgcgcct ttcggcgggt aaattatcga 240

tgagcgtggt gggttatgcc atcgcgtcac actaatccca gaaaagaccc gtccg 295

<210> SEQ ID NO 32
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 32

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agatcatgtt 60

gccactcgt ttaatcacc tgccataaag aaactgttac ccgtaggtag tcacgcaact 120

cgccgcacat ctgaacttca gcctccagta cagcgcggct gaaatcatca ttcatataag 180

tggtcatta gagatagctg atttgtgtag tcggtttatg cagcaacgag acgtcacgga 240

aaatgccgt catccgccac atatcctgat cttcatccca gaaaagaccc gtccg 295

<210> SEQ ID NO 33
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 33

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agattgtagt 60

cgggttatgc agcaaccgcc tcgcggtgat ggtgctgcgc tggagtgaac gcagttatct 120

ggaagatcag gatattgtgc ggatgagcgg cattttccgt gacgtctcgt tgtaatgaaa 180

accgtaatga cagattagcg atttccatgt tgccactcgc tttaatgatg atttcagccg 240

cgctgtactg gaggtgaag ttcagatgtg cggcatccca gaaaagaccc gtccg 295

<210> SEQ ID NO 34
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 34

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agatccgtct 60

-continued

gaatttgacc tgagctcatc cgccacatat cctgatcttc cagataactg ccgtcactcc	120
agcgcagcac catcacgcgc aggcgggttt ctccggcgcg taaaatgcg cttcattaaa	180
attctcatta cagaccactg tcttgccgt aaccgacca gcgccgttg caccacagat	240
gaaacgccga gttaacgcc tcaaaaataa ttcgatccca gaaaagacc gtccg	295

<210> SEQ ID NO 35
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 35

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agattggcgg	60
atgagcggca tttttccagt acagcgcgcg tgaaatcatc attaaagcga gtggcaacat	120
ggaaatcgct gatttgtgta gtcggtttat gcagcaacga gacgtcacgg aatcattagc	180
tcattcatta cacattctga tcttcagat aactgccgtc actccagcgc agcaccatca	240
ccgcgaggcg gttttctccg gcgcgtaaaa atgcatccca gaaaagacc gtccg	295

<210> SEQ ID NO 36
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 36

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agataccatg	60
attacggatt cactctatta cgccagctgg cgaaaggggg atgtgctgca aggcgattaa	120
gttgggtaac gccagggttt tccagtcac gacgttgtaa aacgacggcc agtcattacg	180
taattcatta cactcatgtt tctgtgtga aattgttacc cgctcacaat tccacacaac	240
atacagccg gaagcataaa gtgtaaagcc tgggatccca gaaaagacc gtccg	295

<210> SEQ ID NO 37
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 37

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agatccatca	60
aaaataattc gcgtattacg gtcaatccgc cgtttgttcc caggagaat ccgacgggtt	120
gttactcgct cacatttaat gttgatgaaa gctggctaca ggaaggccag acgtaatgaa	180
tttttaatg agtcaattcg gcgtttcatc tgtgttgcaa cgggcgctgg gtcggttacg	240
gccaggacag tcgtttgccg tctgaatttg acctatccca gaaaagacc gtccg	295

<210> SEQ ID NO 38
 <211> LENGTH: 295
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 38

gtgtgtgata cgaaacgaag cattggaggc attggaattt ctactcttgt agatgggata	60
---	----

-continued

```

ctgacgaaac gcctaattggc ttctgctacc tggagagacg cgcccgctga tcctttgcga    120
atacggccac gcgatgggta acagtcttgg cggtttcgct aaatactggc agtaatgacg    180
tcagtaatga cgacttcagg gcggcttcgt ctgggactgg gtggatcagt cgctgattaa    240
atatgatgaa aacggcaacc cgtggtcggc ttacatccca gaaaagaccg gtccg        295

```

```

<210> SEQ ID NO 39
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NUCLEAR LOCALIZATION SEQUENCE FRAGMENT

```

```

<400> SEQUENCE: 39

```

```

atggcaccca agaagaagag gaaggtgta        30

```

```

<210> SEQ ID NO 40
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NUCLEAR LOCALIZATION PEPTIDE

```

```

<400> SEQUENCE: 40

```

```

Met Ala Pro Lys Lys Lys Arg Lys Val Leu
1           5           10

```

```

<210> SEQ ID NO 41
<211> LENGTH: 199
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

```

```

<400> SEQUENCE: 41

```

```

ggccccaat tctaatttct actgtttag atacgacgtt gaagcttcac aatttttacg    60
ccgacataga ggagaagcat atgtacaatg agccggtcac aacctcgag acacgacgtt    120
gaagcttaac aaacacacca cagacgtggg tcaataccat tgaagatga gaaaagtaac    180
aatatacgcg ctctgccc        199

```

```

<210> SEQ ID NO 42
<211> LENGTH: 195
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

```

```

<400> SEQUENCE: 42

```

```

ggccccaat tctaatttct actgtttag atcttttctc atctttcaat ggtttttgta    60
tcctcgccat ttactctcgt cgggaaagag cgcaatggat acaattcccc actttttctca    120
tcttacaatg gtattgacct acgtctgtgg tgtgtttgtg aagcttcaac gtcgtcaata    180
tacgcgctcc tgccc        195

```

```

<210> SEQ ID NO 43
<211> LENGTH: 201
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

```

```

<400> SEQUENCE: 43

```

-continued

```

ggccccaat tctaatttct actgtttag atccgacgag agtaaatggc gattttttca    60
ataccattga aagatgagaa aagtaaagaa ttgtatccat tgcgtctggt cccgacgaga    120
gtataaggcg aggatacgtt ctctatggag gatggcatag gtgatgaaga tgaaggagaa    180
gcaatatacg cgctcctgcc c                                              201

```

```

<210> SEQ ID NO 44
<211> LENGTH: 201
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

```

```

<400> SEQUENCE: 44

```

```

ggccccaat tctaatttct actgtttag attccacacc tctgaccaac gctttttatt    60
ggatatgattg cccttggtgg tactattggt acaggtcttt tcattggatt atccacacct    120
ctgtaaaacg cgggccagtg gggcgctctt atatcatatt tatttatggg ttctttggca    180
tcaatatacg cgctcctgcc c                                              201

```

```

<210> SEQ ID NO 45
<211> LENGTH: 201
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

```

```

<400> SEQUENCE: 45

```

```

ggccccaat tctaatttct actgtttag atacagtttt ctcacaaaga ttttttttct    60
gtcacgcagt ccttgggtga aatggctaca ttcacccctg ttacatcctc gttcacagtt    120
ttctcataaa gattcctttc tccagcattt ggtgcggcca atgggtacat gtattggttt    180
tcaatatacg cgctcctgcc c                                              201

```

```

<210> SEQ ID NO 46
<211> LENGTH: 197
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

```

```

<400> SEQUENCE: 46

```

```

ggccccaat tctaatttct actgtttag atggtaatta tcacaataat gatttttcat    60
tcaattttgg acgtacaaag ttccactggc ggcattggatt agtatttga aggaattat    120
cacataaatg aacttggtcc ctgtcaaata ttacggtgaa ttcgagttct gggtcgccaa    180
tatacgcgct cctgccc                                              197

```

```

<210> SEQ ID NO 47
<211> LENGTH: 201
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

```

```

<400> SEQUENCE: 47

```

```

ggccccaat tctaatttct actgtttag attggaggat ggcataaggat attttttaat    60
tgtatccatt gcgtctcttc ccgacgagag taaatggcga ggatacgttc tccatggagg    120
atggcatata agatgaagat gaaggagaag tacagaacgc tgaagtgaag agagagctta    180

```

-continued

acaatatacg cgctcctgcc c 201

<210> SEQ ID NO 48
 <211> LENGTH: 198
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 48

ggccccaat tctaatttct actgtttag atttcacttc agcgttctgt actttttcca 60
 atagtaccac caagggaat cataccaata tgtctttgct taagctcccc cttcacttca 120
 gcgttttata cttctccttc atcttcatca cctatgccat cctccataga gaacgtatca 180
 atatacgcg ctcctgccc 198

<210> SEQ ID NO 49
 <211> LENGTH: 201
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 49

ggccccaat tctaatttct actgtttag atacgcagtc cttgggtgaa attttttcca 60
 gtggcgctc ttatatcata ttatttatg ggttctttgg catattcggg cagcgagtcc 120
 ttgggttaaa tggtacatt catcctgtt acatcctctt tcacagtttt ctcacaaaga 180
 tcaatatacg cgctcctgcc c 201

<210> SEQ ID NO 50
 <211> LENGTH: 198
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 50

ggccccaat tctaatttct actgtttag atagaaaact gtgaaagagg attttttaac 60
 caatacatgt aaccattggc cgcaccaaat gctggagaaa ggaatctccc tgagaaaact 120
 gtgaattagg atgtaacagg gatgaatgta gccatttcac ccaaggactg cgtgacagca 180
 atatacgcg ctcctgccc 198

<210> SEQ ID NO 51
 <211> LENGTH: 201
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 51

ggccccaat tctaatttct actgtttag attacaatga gccggtcaca actttttggc 60
 atagcaatga caaattcaaa agaagacgcc gacatagagg agaagcacgg gtacaatgag 120
 ccgtaaacaa ccctctttca cgacgttgaa gcttcacaaa cacaccacag acgtgggtca 180
 acaatatacg cgctcctgcc c 201

<210> SEQ ID NO 52
 <211> LENGTH: 201
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: EDITING CASSETTE SEQUENCE

<400> SEQUENCE: 52

ggccccaat tctaatttct actgtttag atcacacctc tgaccaacgc cgtttttgta 60
tgattgcct tgggtgtact attggtacag gtcttttcat tggtttaagt acacctctga 120
cctaagccgg cccagtgggc gctcttatat catatttatt tatgggttct ttggcatatt 180
ccaatatacg cgctcctgcc c 201

<210> SEQ ID NO 53
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 53

cctcccctag caacgccct t 21

<210> SEQ ID NO 54
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 54

ggatttttac cgtcaccaa a 21

<210> SEQ ID NO 55
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 55

tggtcttatt ctctctccca t 21

<210> SEQ ID NO 56
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 56

gctgaactct gcaggtcagt t 21

<210> SEQ ID NO 57
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 57

tcaggttagc ttcttgtagc t 21

<210> SEQ ID NO 58
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

```

<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 58

agattcagaa ccacttctct a                21

<210> SEQ ID NO 59
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 59

ctgtagtcca aggagggtat a                21

<210> SEQ ID NO 60
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 60

gataagcatg ttttccaaga a                21

<210> SEQ ID NO 61
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 61

ccctttaa gaagctaagt t                21

<210> SEQ ID NO 62
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 62

acttctctaa aaatatggct c                21

<210> SEQ ID NO 63
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 63

tcagattcag aaccacttct c                21

<210> SEQ ID NO 64
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 64

tatggctcta ttctctctcc c                21

```

-continued

<210> SEQ ID NO 65
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 65

tctctcccat cctcaggtta g 21

<210> SEQ ID NO 66
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: SPACER SEQUENCE

<400> SEQUENCE: 66

tcaggtgtat ttgacctac g 21

<210> SEQ ID NO 67
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K535R/N539S

<400> SEQUENCE: 67

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35 40 45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50 55 60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65 70 75 80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85 90 95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
 100 105 110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
 115 120 125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
 130 135 140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
 145 150 155 160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
 165 170 175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
 180 185 190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
 195 200 205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
 210 215 220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
 225 230 235 240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys

-continued

245								250					255				
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu		
			260					265					270				
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys		
			275				280					285					
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu		
			290			295					300						
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys		
305					310					315					320		
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr		
			325						330					335			
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser		
			340					345					350				
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile		
			355				360					365					
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys		
						375					380						
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile		
385					390					395					400		
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys		
				405					410					415			
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu		
			420					425					430				
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu		
			435				440					445					
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala		
		450				455					460						
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp		
465					470					475					480		
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro		
				485					490					495			
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro		
			500					505					510				
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala		
			515				520					525					
Asp	Gly	Trp	Ser	Lys	Ser	Arg	Glu	Tyr	Ser	Ser	Asn	Ala	Ile	Ile	Leu		
		530				535					540						
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys		
545					550					555					560		
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp		
				565					570					575			
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile		
			580					585					590				
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro		
			595				600					605					
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser		
					615						620						
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe		
625					630					635					640		
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp		
			645						650					655			
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu		
			660					665					670				

Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
675 680 685															
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
690 695 700															
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
705 710 715 720															
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile
725 730 735															
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser
740 745 750															
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg
755 760 765															
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
770 775 780															
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe
785 790 795 800															
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys
805 810 815															
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr
820 825 830															
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn
835 840 845															
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr
850 855 860															
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu
865 870 875 880															
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val
885 890 895															
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys
900 905 910															
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys
915 920 925															
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val
930 935 940															
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala
945 950 955 960															
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu
965 970 975															
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn
980 985 990															
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu
995 1000 1005															
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val	
1010 1015 1020															
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr	
1025 1030 1035															
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe	
1040 1045 1050															
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe	
1055 1060 1065															
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr	
1070 1075 1080															

-continued

Phe Asp	Tyr Asn Asn Phe Ile	Thr Gln Asn Thr Val	Met Ser Lys
1085	1090	1095	
Ser Ser	Trp Ser Val Tyr Thr	Tyr Gly Val Arg Ile	Lys Arg Arg
1100	1105	1110	
Phe Val	Asn Gly Arg Phe Ser	Asn Glu Ser Asp Thr	Ile Asp Ile
1115	1120	1125	
Thr Lys	Asp Met Glu Lys Thr	Leu Glu Met Thr Asp	Ile Asn Trp
1130	1135	1140	
Arg Asp	Gly His Asp Leu Arg	Gln Asp Ile Ile Asp	Tyr Glu Ile
1145	1150	1155	
Val Gln	His Ile Phe Glu Ile	Phe Arg Leu Thr Val	Gln Met Arg
1160	1165	1170	
Asn Ser	Leu Ser Glu Leu Glu	Asp Arg Asp Tyr Asp	Arg Leu Ile
1175	1180	1185	
Ser Pro	Val Leu Asn Glu Asn	Asn Ile Phe Tyr Asp	Ser Ala Lys
1190	1195	1200	
Ala Gly	Asp Ala Leu Pro Lys	Asp Ala Asp Ala Asn	Gly Ala Tyr
1205	1210	1215	
Cys Ile	Ala Leu Lys Gly Leu	Tyr Glu Ile Lys Gln	Ile Thr Glu
1220	1225	1230	
Asn Trp	Lys Glu Asp Gly Lys	Phe Ser Arg Asp Lys	Leu Lys Ile
1235	1240	1245	
Ser Asn	Lys Asp Trp Phe Asp	Phe Ile Gln Asn Lys	Arg Tyr Leu
1250	1255	1260	

<210> SEQ ID NO 68

<211> LENGTH: 1060

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT K535R, N539S, K594L, E730Q

<400> SEQUENCE: 68

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser	
1 5 10 15	
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln	
20 25 30	
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly	
35 40 45	
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly	
50 55 60	
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser	
65 70 75 80	
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp	
85 90 95	
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys	
100 105 110	
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile	
115 120 125	
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala	
130 135 140	
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe	
145 150 155 160	
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser	
165 170 175	

Ala 180	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys 185	His	Arg	Ile	Val	Asn 190	Asn	
Ala 195	Glu	Ile	Phe	Phe	Ser	Asn	Ala 200	Leu	Val	Tyr	Arg	Arg 205	Ile	Val	Lys
Ser 210	Leu	Ser	Asn	Asp	Asp	Ile 215	Asn	Lys	Ile	Ser	Gly 220	Asp	Met	Lys	Asp
Ser 225	Leu	Lys	Glu	Met	Ser 230	Leu	Glu	Glu	Ile	Tyr 235	Ser	Tyr	Glu	Lys	Tyr 240
Gly	Glu	Phe	Ile	Thr 245	Gln	Glu	Gly	Ile	Ser 250	Phe	Tyr	Asn	Asp	Ile 255	Cys
Gly	Lys	Val	Asn 260	Ser	Phe	Met	Asn	Leu 265	Tyr	Cys	Gln	Lys	Asn 270	Lys	Glu
Asn 275	Lys	Asn	Leu	Tyr	Lys	Leu	Gln 280	Lys	Leu	His	Lys	Gln 285	Ile	Leu	Cys
Ile 290	Ala	Asp	Thr	Ser	Tyr 295	Glu	Val	Pro	Tyr	Lys	Phe 300	Glu	Ser	Asp	Glu
Glu 305	Val	Tyr	Gln	Ser	Val 310	Asn	Gly	Phe	Leu	Asp 315	Asn	Ile	Ser	Ser	Lys 320
His	Ile	Val	Glu 325	Arg	Leu	Arg	Lys	Ile	Gly 330	Asp	Asn	Tyr	Asn	Gly 335	Tyr
Asn	Leu	Asp	Lys 340	Ile	Tyr	Ile	Val	Ser 345	Lys	Phe	Tyr	Glu	Ser 350	Val	Ser
Gln	Lys	Thr 355	Tyr	Arg	Asp	Trp	Glu 360	Thr	Ile	Asn	Thr	Ala 365	Leu	Glu	Ile
His 370	Tyr	Asn	Asn	Ile	Leu 375	Pro	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Asp	Lys
Val 385	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys 395	Ser	Ile	Thr	Glu	Ile 400
Asn	Glu	Leu	Val	Ser 405	Asn	Tyr	Lys	Leu	Cys 410	Ser	Asp	Asp	Asn	Ile	Lys 415
Ala	Glu	Thr	Tyr 420	Ile	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn 430	Asn	Phe	Glu
Ala	Gln	Glu	Leu	Lys 435	Tyr	Asn	Pro 440	Glu	Ile	His	Leu	Val 445	Glu	Ser	Glu
Leu 450	Lys	Ala	Ser	Glu	Leu 455	Asn	Val	Leu	Asp	Val 460	Ile	Met	Asn	Ala	
Phe 465	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu 475	Leu	Val	Asp	Lys	Asp 480
Asn	Asn	Phe	Tyr	Ala 485	Glu	Leu	Glu	Glu	Ile 490	Tyr	Asp	Glu	Ile	Tyr	Pro 495
Val	Ile	Ser	Leu	Tyr	Asn 500	Leu	Val	Arg 505	Asn	Tyr	Val	Thr 510	Gln	Lys	Pro
Tyr	Ser	Thr 515	Lys	Lys	Ile	Lys	Leu 520	Asn	Phe	Gly	Ile	Pro 525	Thr	Leu	Ala
Asp	Gly 530	Trp	Ser	Lys	Ser	Arg 535	Glu	Tyr	Ser	Ser	Asn 540	Ala	Ile	Ile	Leu
Met 545	Arg	Asp	Asn	Leu	Tyr 550	Tyr	Leu	Gly	Ile	Phe 555	Asn	Ala	Lys	Asn	Lys 560
Pro	Asp	Lys	Lys	Ile 565	Ile	Glu	Gly	Asn	Thr 570	Ser	Glu	Asn	Lys	Gly 575	Asp
Tyr	Lys	Lys	Met 580	Ile	Tyr	Asn	Leu	Leu 585	Pro	Gly	Pro	Asn 590	Lys	Met	Ile
Pro	Leu	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro

-continued

595	600	605
Ser Ala Tyr Ile Leu Glu Gly 610	Tyr Lys Gln Asn 615	Lys His Ile Lys Ser 620
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp 625	Leu Ile Asp Tyr Phe 630	635 640
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp 645	650	655
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu 660	665	670
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys 675	680	685
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile 690	695	700
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His 705	710	715 720
Thr Met Tyr Leu Lys Asn Leu Phe Ser Gln Glu Asn Leu Lys Asp Ile 725	730	735
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser 740	745	750
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg 755	760	765
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val 770	775	780
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe 785	790	795 800
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys 805	810	815
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr 820	825	830
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn 835	840	845
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr 850	855	860
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu 865	870	875 880
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val 885	890	895
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys 900	905	910
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys 915	920	925
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val 930	935	940
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala 945	950	955 960
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu 965	970	975
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn 980	985	990
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu 995	1000	1005
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val 1010	1015	1020

-continued

Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
1025 1030 1035

Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
1040 1045 1050

Lys Asp Leu Thr Val Asp Ala
1055 1060

<210> SEQ ID NO 69

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT K95L

<400> SEQUENCE: 69

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
20 25 30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
35 40 45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
50 55 60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
65 70 75 80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Leu Asp
85 90 95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
100 105 110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
115 120 125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
130 135 140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
145 150 155 160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
165 170 175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
180 185 190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
195 200 205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
210 215 220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
225 230 235 240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
245 250 255

Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
260 265 270

Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys
275 280 285

Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu
290 295 300

Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys
305 310 315 320

His 325	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
His 370	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
Val 385	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
Phe 465	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
Met 545	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
Ser 610	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
Ser 625	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
Val 675	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
Asp 690	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
Tyr 705	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile

-continued

Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	740	745	750
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	755	760	765
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	770	775	780
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	785	790	795
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	805	810	815
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	820	825	830
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	835	840	845
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr	850	855	860
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	865	870	875
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val	885	890	895
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys	900	905	910
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys	915	920	925
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val	930	935	940
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala	945	950	955
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu	965	970	975
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn	980	985	990
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu	995	1000	1005
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val		1010	1015	1020
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr		1025	1030	1035
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe		1040	1045	1050
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe		1055	1060	1065
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr		1070	1075	1080
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys		1085	1090	1095
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg		1100	1105	1110
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile		1115	1120	1125
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp		1130	1135	1140
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile				

-continued

1145	1150	1155
Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg		
1160	1165	1170
Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile		
1175	1180	1185
Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys		
1190	1195	1200
Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr		
1205	1210	1215
Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu		
1220	1225	1230
Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile		
1235	1240	1245
Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu		
1250	1255	1260

<210> SEQ ID NO 70

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT V207I, K278T

<400> SEQUENCE: 70

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser		
1	5	10
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln		
20	25	30
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly		
35	40	45
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly		
50	55	60
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser		
65	70	75
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp		
85	90	95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys		
100	105	110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile		
115	120	125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala		
130	135	140
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe		
145	150	155
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser		
165	170	175
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn		
180	185	190
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Ile Lys		
195	200	205
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp		
210	215	220
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr		
225	230	235
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys		

-continued

245								250					255				
Gly	Lys	Val	Asn 260	Ser	Phe	Met	Asn	Leu 265	Tyr	Cys	Gln	Lys	Asn 270	Lys	Glu		
Asn	Lys	Asn 275	Leu	Tyr	Thr	Leu	Gln 280	Lys	Leu	His	Lys	Gln 285	Ile	Leu	Cys		
Ile	Ala	Asp	Thr	Ser	Tyr	Glu 295	Val	Pro	Tyr	Lys	Phe 300	Glu	Ser	Asp	Glu		
Glu 305	Val	Tyr	Gln	Ser	Val 310	Asn	Gly	Phe	Leu	Asp 315	Asn	Ile	Ser	Ser	Lys 320		
His	Ile	Val	Glu 325	Arg	Leu	Arg	Lys	Ile 330	Gly	Asp	Asn	Tyr	Asn	Gly 335	Tyr		
Asn	Leu	Asp	Lys 340	Ile	Tyr	Ile	Val	Ser 345	Lys	Phe	Tyr	Glu	Ser 350	Val	Ser		
Gln	Lys	Thr 355	Tyr	Arg	Asp	Trp	Glu 360	Thr	Ile	Asn	Thr	Ala 365	Leu	Glu	Ile		
His	Tyr 370	Asn	Asn	Ile	Leu	Pro 375	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Asp	Lys		
Val 385	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys 395	Ser	Ile	Thr	Glu	Ile 400		
Asn	Glu	Leu	Val	Ser 405	Asn	Tyr	Lys	Leu	Cys 410	Ser	Asp	Asp	Asn	Ile	Lys		
Ala	Glu	Thr	Tyr 420	Ile	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn 430	Asn	Phe	Glu		
Ala	Gln	Glu 435	Leu	Lys	Tyr	Asn	Pro 440	Glu	Ile	His	Leu	Val 445	Glu	Ser	Glu		
Leu	Lys 450	Ala	Ser	Glu	Leu	Lys 455	Asn	Val	Leu	Asp	Val 460	Ile	Met	Asn	Ala		
Phe 465	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu 475	Leu	Val	Asp	Lys	Asp 480		
Asn	Asn	Phe	Tyr 485	Ala	Glu	Leu	Glu	Glu 490	Ile	Tyr	Asp	Glu	Ile	Tyr 495	Pro		
Val	Ile	Ser	Leu 500	Tyr	Asn	Leu	Val	Arg 505	Asn	Tyr	Val	Thr 510	Gln	Lys	Pro		
Tyr	Ser	Thr 515	Lys	Lys	Ile	Lys	Leu 520	Asn	Phe	Gly	Ile	Pro 525	Thr	Leu	Ala		
Asp	Gly 530	Trp	Ser	Lys	Ser	Lys 535	Glu	Tyr	Ser	Asn	Asn 540	Ala	Ile	Ile	Leu		
Met 545	Arg	Asp	Asn	Leu	Tyr 550	Tyr	Leu	Gly	Ile	Phe 555	Asn	Ala	Lys	Asn	Lys 560		
Pro	Asp	Lys	Lys 565	Ile	Ile	Glu	Gly	Asn	Thr 570	Ser	Glu	Asn	Lys	Gly 575	Asp		
Tyr	Lys	Lys	Met 580	Ile	Tyr	Asn	Leu	Leu 585	Pro	Gly	Pro	Asn 590	Lys	Met	Ile		
Pro	Lys	Val 595	Phe	Leu	Ser	Ser	Lys 600	Thr	Gly	Val	Glu	Thr 605	Tyr	Lys	Pro		
Ser	Ala 610	Tyr	Ile	Leu	Glu	Gly 615	Tyr	Lys	Gln	Asn	Lys 620	His	Ile	Lys	Ser		
Ser 625	Lys	Asp	Phe	Asp	Ile 630	Thr	Phe	Cys	His	Asp 635	Leu	Ile	Asp	Tyr	Phe 640		
Lys	Asn	Cys	Ile 645	Ala	Ile	His	Pro	Glu	Trp 650	Lys	Asn	Phe	Gly 655	Phe	Asp		
Phe	Ser	Asp	Thr 660	Ser	Thr	Tyr	Glu	Asp 665	Ile	Ser	Gly	Phe 670	Tyr	Arg	Glu		

Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	
	675						680					685				
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	
	690					695					700					
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	
	705				710					715					720	
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	
			725						730					735		
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	
			740					745					750			
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	
	755						760					765				
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	
	770					775					780					
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	
	785				790					795					800	
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	
			805						810					815		
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	
			820					825					830			
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	
		835					840					845				
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr	
	850					855					860					
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	
	865				870					875					880	
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val	
			885						890					895		
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys	
			900					905					910			
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys	
			915				920					925				
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val	
	930					935					940					
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala	
	945				950					955					960	
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu	
			965						970					975		
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn	
			980					985					990			
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu	
	995					1000						1005				
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr										

-continued

Phe Asp	Tyr Asn Asn Phe Ile	Thr Gln Asn Thr Val	Met Ser Lys
1085	1090	1095	
Ser Ser	Trp Ser Val Tyr Thr	Tyr Gly Val Arg Ile	Lys Arg Arg
1100	1105	1110	
Phe Val	Asn Gly Arg Phe Ser	Asn Glu Ser Asp Thr	Ile Asp Ile
1115	1120	1125	
Thr Lys	Asp Met Glu Lys Thr	Leu Glu Met Thr Asp	Ile Asn Trp
1130	1135	1140	
Arg Asp	Gly His Asp Leu Arg	Gln Asp Ile Ile Asp	Tyr Glu Ile
1145	1150	1155	
Val Gln	His Ile Phe Glu Ile	Phe Arg Leu Thr Val	Gln Met Arg
1160	1165	1170	
Asn Ser	Leu Ser Glu Leu Glu	Asp Arg Asp Tyr Asp	Arg Leu Ile
1175	1180	1185	
Ser Pro	Val Leu Asn Glu Asn	Asn Ile Phe Tyr Asp	Ser Ala Lys
1190	1195	1200	
Ala Gly	Asp Ala Leu Pro Lys	Asp Ala Asp Ala Asn	Gly Ala Tyr
1205	1210	1215	
Cys Ile	Ala Leu Lys Gly Leu	Tyr Glu Ile Lys Gln	Ile Thr Glu
1220	1225	1230	
Asn Trp	Lys Glu Asp Gly Lys	Phe Ser Arg Asp Lys	Leu Lys Ile
1235	1240	1245	
Ser Asn	Lys Asp Trp Phe Asp	Phe Ile Gln Asn Lys	Arg Tyr Leu
1250	1255	1260	

<210> SEQ ID NO 71
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K511D

<400> SEQUENCE: 71

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser	
1 5 10 15	
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln	
20 25 30	
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly	
35 40 45	
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly	
50 55 60	
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser	
65 70 75 80	
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp	
85 90 95	
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys	
100 105 110	
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile	
115 120 125	
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala	
130 135 140	
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe	
145 150 155 160	
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser	
165 170 175	

Ala 180	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys 185	His	Arg	Ile	Val	Asn 190	Asn
Ala 195	Glu	Ile	Phe	Phe	Ser	Asn	Ala 200	Leu	Val	Tyr	Arg	Arg 205	Ile	Lys
Ser 210	Leu	Ser	Asn	Asp	Asp	Ile 215	Asn	Lys	Ile	Ser	Gly 220	Asp	Met	Asp
Ser 225	Leu	Lys	Glu	Met	Ser 230	Leu	Glu	Glu	Ile	Tyr 235	Ser	Tyr	Glu	Tyr 240
Gly	Glu	Phe	Ile	Thr 245	Gln	Glu	Gly	Ile	Ser 250	Phe	Tyr	Asn	Asp	Cys 255
Gly	Lys	Val	Asn 260	Ser	Phe	Met	Asn	Leu 265	Tyr	Cys	Gln	Lys	Asn 270	Glu
Asn 275	Lys	Asn	Leu	Tyr	Lys	Leu	Gln 280	Lys	Leu	His	Lys	Gln 285	Ile	Cys
Ile 290	Ala	Asp	Thr	Ser	Tyr 295	Glu	Val	Pro	Tyr	Lys	Phe 300	Glu	Ser	Glu
Glu 305	Val	Tyr	Gln	Ser	Val 310	Asn	Gly	Phe	Leu	Asp 315	Asn	Ile	Ser	Lys 320
His	Ile	Val	Glu 325	Arg	Leu	Arg	Lys	Ile	Gly 330	Asp	Asn	Tyr	Asn 335	Tyr
Asn	Leu	Asp	Lys 340	Ile	Tyr	Ile	Val	Ser 345	Lys	Phe	Tyr	Glu	Ser 350	Ser
Gln	Lys	Thr 355	Tyr	Arg	Asp	Trp	Glu 360	Thr	Ile	Asn	Thr	Ala 365	Leu	Ile
His 370	Tyr	Asn	Asn	Ile	Leu 375	Pro	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Lys
Val 385	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys 395	Ser	Ile	Thr	Ile 400
Asn	Glu	Leu	Val	Ser 405	Asn	Tyr	Lys	Leu	Cys 410	Ser	Asp	Asp	Asn 415	Lys
Ala	Glu	Thr	Tyr 420	Ile	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn 430	Asn	Glu
Ala	Gln	Glu	Leu 435	Lys	Tyr	Asn	Pro 440	Glu	Ile	His	Leu	Val 445	Glu	Glu
Leu 450	Lys	Ala	Ser	Glu	Leu 455	Lys	Asn	Val	Leu	Asp	Val 460	Ile	Met	Ala
Phe 465	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu 475	Leu	Val	Asp	Asp 480
Asn	Asn	Phe	Tyr 485	Ala	Glu	Leu	Glu	Glu 490	Ile	Tyr	Asp	Glu	Ile 495	Pro
Val	Ile	Ser	Leu 500	Tyr	Asn	Leu	Val	Arg 505	Asn	Tyr	Val	Thr 510	Gln	Pro
Tyr	Ser	Thr 515	Lys	Lys	Ile	Lys	Leu 520	Asn	Phe	Gly	Ile	Pro 525	Thr	Ala
Asp	Gly 530	Trp	Ser	Lys	Ser	Lys 535	Glu	Tyr	Ser	Asn	Asn 540	Ala	Ile	Leu
Met 545	Arg	Asp	Asn	Leu	Tyr 550	Tyr	Leu	Gly	Ile	Phe 555	Asn	Ala	Lys	Lys 560
Pro	Asp	Lys	Lys 565	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Asp 575
Tyr	Lys	Lys	Met 580	Ile	Tyr	Asn	Leu	Leu 585	Pro	Gly	Pro	Asn	Lys 590	Ile
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Pro

-continued

595	600	605
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser 610 615 620		
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe 625 630 635 640		
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp 645 650 655		
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu 660 665 670		
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys 675 680 685		
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile 690 695 700		
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His 705 710 715 720		
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile 725 730 735		
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser 740 745 750		
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg 755 760 765		
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val 770 775 780		
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe 785 790 795 800		
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys 805 810 815		
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr 820 825 830		
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn 835 840 845		
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr 850 855 860		
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu 865 870 875 880		
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val 885 890 895		
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys 900 905 910		
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys 915 920 925		
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val 930 935 940		
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala 945 950 955 960		
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu 965 970 975		
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn 980 985 990		
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu 995 1000 1005		
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val 1010 1015 1020		

Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr
	1025					1030					1035			
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe
	1040					1045					1050			
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe
	1055					1060					1065			
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr
	1070					1075					1080			
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys
	1085					1090					1095			
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg
	1100					1105					1110			
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile
	1115					1120					1125			
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp
	1130					1135					1140			
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile
	1145					1150					1155			
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg
	1160					1165					1170			
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile
	1175					1180					1185			
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys
	1190					1195					1200			
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr
	1205					1210					1215			
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu
	1220					1225					1230			
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile
	1235					1240					1245			
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu
	1250					1255					1260			

<400> SEQUENCE: 72

Met 1	Asn	Asn	Gly	Thr 5	Asn	Asn	Phe	Gln	Asn 10	Phe	Ile	Gly	Ile	Ser 15	Ser
Leu	Gln	Lys	Thr 20	Leu	Arg	Asn	Ala	Leu 25	Ile	Pro	Thr	Glu	Thr 30	Thr	Gln
Gln	Phe	Ile	Val 35	Lys	Asn	Gly	Ile 40	Ile	Lys	Glu	Asp	Glu 45	Leu	Arg	Gly
Glu	Asn	Arg	Gln	Ile	Leu	Lys 55	Asp	Ile	Met	Asp	Asp 60	Tyr	Tyr	Arg	Gly
Phe 65	Ile	Ser	Glu	Thr 70	Leu	Ser	Ser	Ile	Asp	Asp 75	Ile	Asp	Trp	Thr	Ser 80
Leu	Phe	Glu	Lys 85	Met	Glu	Ile	Gln	Leu	Lys 90	Asn	Gly	Asp	Asn 95	Lys	Asp
Thr	Leu	Ile	Lys 100	Glu	Gln	Thr	Glu	Tyr 105	Arg	Lys	Ala	Ile 110	His	Lys	Lys

Phe	Ala	Asn	Asp	Asp	Arg	Phe	Lys	Asn	Met	Phe	Ser	Ala	Lys	Leu	Ile
	115						120					125			
Ser	Asp	Ile	Leu	Pro	Glu	Phe	Val	Ile	His	Asn	Asn	Asn	Tyr	Ser	Ala
	130					135					140				
Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe
	145				150					155					160
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser
				165					170					175	
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn
			180					185					190		
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys
		195					200					205			
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp
	210					215					220				
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr
	225				230					235					240
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys
				245					250					255	
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu
			260					265					270		
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys
		275					280					285			
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu
	290					295					300				
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys
	305				310					315					320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
				325					330					335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
			340					345					350		
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
		355					360					365			
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
	370					375					380				
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
	385				390					395					400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
				405					410					415	
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
			420					425				430			
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
			435				440					445			
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
		450				455					460				
Phe	His														

-continued

Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
530						535				540					
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
545				550						555					560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
			565					570						575	
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	His	Lys	Met	Ile
			580					585					590		
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
		595					600					605			
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
610					615						620				
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
625				630						635					640
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
			645					650						655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
			660					665					670		
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
		675					680					685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
690					695						700				
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
705				710						715					720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile
			725						730					735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser
			740					745					750		
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg
		755					760					765			
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
770					775						780				
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe
785				790						795					800
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys
			805						810					815	
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr
			820					825					830		
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn
			835				840					845			
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr
850					855						860				
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu
865				870						875					880
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val
			885					890						895	
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys
			900					905					910		
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys
			915				920					925			
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val
930					935						940				
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala

945				950				955				960			
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu
				965				970				975			
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn
				980				985				990			
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu
				995				1000				1005			
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val	
				1010				1015				1020			
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr	
				1025				1030				1035			
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe	
				1040				1045				1050			
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe	
				1055				1060				1065			
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr	
				1070				1075				1080			
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys	
				1085				1090				1095			
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg	
				1100				1105				1110			
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile	
				1115				1120				1125			
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp	
				1130				1135				1140			
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile	
				1145				1150				1155			
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg	
				1160				1165				1170			
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile	
				1175				1180				1185			
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys	
				1190				1195				1200			
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr	
				1205				1210				1215			
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu	
				1220				1225				1230			
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile	
				1235				1240				1245			
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu	
				1250				1255				1260			

<400> SEQUENCE: 73

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
20 25 30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly

-continued

35	40	45
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly		
50	55	60
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser		
65	70	75
80		
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp		
85	90	95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys		
100	105	110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile		
115	120	125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala		
130	135	140
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe		
145	150	155
160		
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser		
165	170	175
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn		
180	185	190
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys		
195	200	205
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp		
210	215	220
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr		
225	230	235
240		
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys		
245	250	255
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu		
260	265	270
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys		
275	280	285
Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu		
290	295	300
Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys		
305	310	315
320		
His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr		
325	330	335
Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser		
340	345	350
Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile		
355	360	365
His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys		
370	375	380
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile		
385	390	395
400		
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys		
405	410	415
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu		
420	425	430
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu		
435	440	445
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala		
450	455	460

-continued

Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp	
465					470					475					480	
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro	
				485					490					495		
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro	
			500					505					510			
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	
		515					520					525				
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	
	530					535					540					
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	
545				550						555					560	
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp	
				565				570						575		
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile	
			580					585					590			
Pro	Lys	Val	Phe	Val	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro	
		595					600					605				
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser	
	610					615					620					
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe	
625					630					635					640	
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp	
			645					650						655		
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu	
			660					665					670			
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	
		675					680					685				
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	
	690					695					700					
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	
705					710					715					720	
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	
			725					730						735		
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	
			740					745					750			
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	
	755						760					765				
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	
	770					775					780					
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	
785				790						795					800	
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	
			805					810						815		
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	
			820					825					830			
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	
		835						840					845			
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr	
	850					855					860					
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	
865					870					875					880	

-continued

Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val
 885 890 895
 Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys
 900 905 910
 Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925
 Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940
 Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960
 Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975
 Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990
 Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005
 Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020
 Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035
 Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050
 Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065
 Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080
 Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095
 Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110
 Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125
 Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140
 Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155
 Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170
 Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185
 Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200
 Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215
 Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230
 Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245
 Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 74

<211> LENGTH: 1263

<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT K712V

<400> SEQUENCE: 74

```

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1          5          10          15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20          25          30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35          40          45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50          55          60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65          70          75          80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85          90          95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
100          105          110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
115          120          125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
130          135          140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
145          150          155          160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
165          170          175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
180          185          190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
195          200          205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
210          215          220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
225          230          235          240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
245          250          255

Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
260          265          270

Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys
275          280          285

Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu
290          295          300

Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys
305          310          315          320

His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr
325          330          335

Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser
340          345          350

Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile
355          360          365

His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys
370          375          380

Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile

```

-continued

385	390	395	400
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys	405	410	415
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu	420	425	430
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu	435	440	445
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala	450	455	460
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp	465	470	475
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro	485	490	495
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln Lys Pro	500	505	510
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala	515	520	525
Asp Gly Trp Ser Lys Ser Lys Glu Tyr Ser Asn Asn Ala Ile Ile Leu	530	535	540
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys	545	550	555
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp	565	570	575
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro Asn Lys Met Ile	580	585	590
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro	595	600	605
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser	610	615	620
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe	625	630	635
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp	645	650	655
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu	660	665	670
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys	675	680	685
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile	690	695	700
Tyr Asn Lys Asp Phe Ser Lys Val Ser Thr Gly Asn Asp Asn Leu His	705	710	715
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile	725	730	735
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser	740	745	750
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg	755	760	765
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val	770	775	780
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe	785	790	795
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys	805	810	815

-continued

Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr
 820 825 830

Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn
 835 840 845

Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr
 850 855 860

Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu
 865 870 875 880

Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val
 885 890 895

Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys
 900 905 910

Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925

Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940

Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960

Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975

Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990

Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005

Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020

Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035

Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050

Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065

Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080

Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095

Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110

Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125

Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140

Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155

Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170

Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185

Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200

Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215

-continued

Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
1220 1225 1230

Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
1235 1240 1245

Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
1250 1255 1260

<210> SEQ ID NO 75
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT E743I

<400> SEQUENCE: 75

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
20 25 30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
35 40 45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
50 55 60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
65 70 75 80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
85 90 95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
100 105 110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
115 120 125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
130 135 140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
145 150 155 160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
165 170 175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
180 185 190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
195 200 205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
210 215 220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
225 230 235 240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
245 250 255

Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
260 265 270

Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys
275 280 285

Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu
290 295 300

Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys
305 310 315 320

His 325	Ile	Val	Glu	Arg 325	Leu	Arg	Lys	Ile	Gly 330	Asp	Asn	Tyr	Asn 335	Gly 335	Tyr
Asn	Leu	Asp	Lys 340	Ile	Tyr	Ile	Val	Ser 345	Lys	Phe	Tyr	Glu	Ser 350	Val	Ser
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala 365	Leu	Glu	Ile
His 370	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Asp	Lys
Val 385	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys 395	Ser	Ile	Thr	Glu	Ile 400
Asn	Glu	Leu	Val	Ser 405	Asn	Tyr	Lys	Leu	Cys 410	Ser	Asp	Asp	Asn	Ile	Lys
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn 430	Asn	Phe	Glu
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val 445	Glu	Ser	Glu
Leu 450	Lys	Ala	Ser	Glu	Leu	Lys 455	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
Phe 465	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp 480
Asn	Asn	Phe	Tyr	Ala 485	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg 505	Asn	Tyr	Val	Thr	Gln 510	Lys	Pro
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn 520	Phe	Gly	Ile	Pro 525	Thr	Leu	Ala
Asp 530	Gly	Trp	Ser	Lys	Ser	Lys 535	Glu	Tyr	Ser	Asn	Asn 540	Ala	Ile	Ile	Leu
Met 545	Arg	Asp	Asn	Leu	Tyr 550	Tyr	Leu	Gly	Ile	Phe 555	Asn	Ala	Lys	Asn	Lys 560
Pro	Asp	Lys	Lys	Ile 565	Ile	Glu	Gly	Asn	Thr 570	Ser	Glu	Asn	Lys	Gly 575	Asp
Tyr	Lys	Lys	Met 580	Ile	Tyr	Asn	Leu	Leu 585	Pro	Gly	Pro	Asn 590	Lys	Met	Ile
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys 600	Thr	Gly	Val	Glu	Thr 605	Tyr	Lys	Pro
Ser 610	Ala	Tyr	Ile	Leu	Glu	Gly 615	Tyr	Lys	Gln	Asn	Lys 620	His	Ile	Lys	Ser
Ser 625	Lys	Asp	Phe	Asp	Ile 630	Thr	Phe	Cys	His	Asp 635	Leu	Ile	Asp	Tyr	Phe 640
Lys	Asn	Cys	Ile	Ala 645	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp 655
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp 665	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile 680	Asp	Trp	Thr	Tyr	Ile 685	Ser	Glu	Lys
Asp 690	Ile	Asp	Leu	Leu	Gln	Glu 695	Lys	Gly	Gln	Leu	Tyr 700	Leu	Phe	Gln	Ile
Tyr 705	Asn	Lys	Asp	Phe	Ser 710	Lys	Lys	Ser	Thr	Gly 715	Asn	Asp	Asn	Leu	His 720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile 735
Val	Leu	Lys	Leu	Asn	Gly	Ile	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser

-continued

740	745	750
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg 755 760 765		
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val 770 775 780		
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe 785 790 795 800		
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys 805 810 815		
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr 820 825 830		
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn 835 840 845		
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr 850 855 860		
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu 865 870 875 880		
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val 885 890 895		
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys 900 905 910		
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys 915 920 925		
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val 930 935 940		
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala 945 950 955 960		
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu 965 970 975		
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn 980 985 990		
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu 995 1000 1005		
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val 1010 1015 1020		
Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr 1025 1030 1035		
Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe 1040 1045 1050		
Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe 1055 1060 1065		
Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr 1070 1075 1080		
Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys 1085 1090 1095		
Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg 1100 1105 1110		
Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile 1115 1120 1125		
Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp 1130 1135 1140		
Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile 1145 1150 1155		

-continued

Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu	
		260						265					270			
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys	
		275					280					285				
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu	
	290					295					300					
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys	
305				310						315					320	
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr	
			325						330					335		
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser	
		340						345					350			
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile	
		355					360					365				
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys	
	370					375					380					
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile	
385					390					395					400	
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys	
			405						410					415		
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu	
		420						425					430			
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu	
	435						440					445				
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala	
	450					455					460					
Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp	
465					470					475					480	
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro	
		485						490						495		
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Lys	Pro	
		500						505					510			
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	
		515					520					525				
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	
	530					535					540					
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	
545					550					555					560	
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp	
		565						570					575			
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	Asn	Lys	Met	Ile	
		580						585					590			
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro	
		595					600					605				
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser	
	610					615					620					
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe	
625					630					635					640	
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp	
		645						650						655		
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu	
		660						665						670		

-continued

Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	675	680	685
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	690	695	700
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	705	710	715
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	725	730	735
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	740	745	750
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	755	760	765
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	770	775	780
Arg	Ser	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	785	790	795
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	805	810	815
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	820	825	830
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	835	840	845
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr	850	855	860
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	865	870	875
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val	885	890	895
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys	900	905	910
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys	915	920	925
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val	930	935	940
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala	945	950	955
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu	965	970	975
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn	980	985	990
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu	995	1000	1005
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val		1010	1015	1020
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr		1025	1030	1035
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe		1040	1045	1050
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe		1055	1060	1065
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr		1070	1075	1080
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys				

-continued

1085	1090	1095
Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg		
1100	1105	1110
Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile		
1115	1120	1125
Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp		
1130	1135	1140
Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile		
1145	1150	1155
Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg		
1160	1165	1170
Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile		
1175	1180	1185
Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys		
1190	1195	1200
Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr		
1205	1210	1215
Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu		
1220	1225	1230
Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile		
1235	1240	1245
Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu		
1250	1255	1260

<210> SEQ ID NO 77

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT : K853R

<400> SEQUENCE: 77

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser		
1	5	10
Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln		
20	25	30
Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly		
35	40	45
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly		
50	55	60
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser		
65	70	75
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp		
85	90	95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys		
100	105	110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile		
115	120	125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala		
130	135	140
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe		
145	150	155
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser		
165	170	175
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn		

-continued

180	185	190
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys 195 200 205		
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp 210 215 220		
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr 225 230 235 240		
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys 245 250 255		
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu 260 265 270		
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys 275 280 285		
Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu 290 295 300		
Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys 305 310 315 320		
His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr 325 330 335		
Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser 340 345 350		
Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile 355 360 365		
His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys 370 375 380		
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile 385 390 395 400		
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys 405 410 415		
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu 420 425 430		
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu 435 440 445		
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala 450 455 460		
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp 465 470 475 480		
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro 485 490 495		
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln Lys Pro 500 505 510		
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala 515 520 525		
Asp Gly Trp Ser Lys Ser Lys Glu Tyr Ser Asn Asn Ala Ile Ile Leu 530 535 540		
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys 545 550 555 560		
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp 565 570 575		
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro Asn Lys Met Ile 580 585 590		
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro 595 600 605		

-continued

Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser
 610 615 620
 Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe
 625 630 635 640
 Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp
 645 650 655
 Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu
 660 665 670
 Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys
 675 680 685
 Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile
 690 695 700
 Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His
 705 710 715 720
 Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile
 725 730 735
 Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser
 740 745 750
 Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg
 755 760 765
 Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val
 770 775 780
 Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe
 785 790 795 800
 Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys
 805 810 815
 Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr
 820 825 830
 Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn
 835 840 845
 Phe Lys Ala Asn Arg Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr
 850 855 860
 Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu
 865 870 875 880
 Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val
 885 890 895
 Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys
 900 905 910
 Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925
 Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940
 Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960
 Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975
 Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990
 Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005
 Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020

-continued

Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr
1025						1030					1035			
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe
1040						1045					1050			
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe
1055						1060					1065			
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr
1070						1075					1080			
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys
1085						1090					1095			
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg
1100						1105					1110			
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile
1115						1120					1125			
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp
1130						1135					1140			
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile
1145						1150					1155			
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg
1160						1165					1170			
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile
1175						1180					1185			
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys
1190						1195					1200			
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr
1205						1210					1215			
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu
1220						1225					1230			
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile
1235						1240					1245			
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu
1250						1255					1260			

<210> SEQ ID NO 78

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT R1113F

<400> SEQUENCE: 78

Met	Asn	Asn	Gly	Thr	Asn	Asn	Phe	Gln	Asn	Phe	Ile	Gly	Ile	Ser	Ser
1				5					10				15		
Leu	Gln	Lys	Thr	Leu	Arg	Asn	Ala	Leu	Ile	Pro	Thr	Glu	Thr	Thr	Gln
			20				25						30		
Gln	Phe	Ile	Val	Lys	Asn	Gly	Ile	Ile	Lys	Glu	Asp	Glu	Leu	Arg	Gly
			35			40					45				
Glu	Asn	Arg	Gln	Ile	Leu	Lys	Asp	Ile	Met	Asp	Asp	Tyr	Tyr	Arg	Gly
			50			55				60					
Phe	Ile	Ser	Glu	Thr	Leu	Ser	Ser	Ile	Asp	Asp	Ile	Asp	Trp	Thr	Ser
65					70					75				80	
Leu	Phe	Glu	Lys	Met	Glu	Ile	Gln	Leu	Lys	Asn	Gly	Asp	Asn	Lys	Asp
			85					90						95	
Thr	Leu	Ile	Lys	Glu	Gln	Thr	Glu	Tyr	Arg	Lys	Ala	Ile	His	Lys	Lys
			100					105						110	

-continued

Phe 115	Ala	Asn	Asp	Asp	Arg	Phe	Lys 120	Asn	Met	Phe	Ser	Ala 125	Lys	Leu	Ile
Ser 130	Asp	Ile	Leu	Pro	Glu	Phe 135	Val	Ile	His	Asn	Asn 140	Asn	Tyr	Ser	Ala
Ser 145	Glu	Lys	Glu	Glu	Lys 150	Thr	Gln	Val	Ile	Lys 155	Leu	Phe	Ser	Arg	Phe 160
Ala	Thr	Ser	Phe	Lys 165	Asp	Tyr	Phe	Lys	Asn 170	Arg	Ala	Asn	Cys	Phe	Ser 175
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys 185	His	Arg	Ile	Val	Asn 190	Asp	Asn
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala 200	Leu	Val	Tyr	Arg	Arg 205	Ile	Val	Lys
Ser 210	Leu	Ser	Asn	Asp	Asp	Ile 215	Asn	Lys	Ile	Ser	Gly 220	Asp	Met	Lys	Asp
Ser 225	Leu	Lys	Glu	Met	Ser 230	Leu	Glu	Glu	Ile	Tyr 235	Ser	Tyr	Glu	Lys	Tyr 240
Gly	Glu	Phe	Ile	Thr 245	Gln	Glu	Gly	Ile	Ser 250	Phe	Tyr	Asn	Asp	Ile	Cys 255
Gly	Lys	Val	Asn 260	Ser	Phe	Met	Asn	Leu 265	Tyr	Cys	Gln	Lys	Asn 270	Lys	Glu
Asn	Lys	Asn 275	Leu	Tyr	Lys	Leu	Gln 280	Lys	Leu	His	Lys	Gln 285	Ile	Leu	Cys
Ile 290	Ala	Asp	Thr	Ser	Tyr	Glu 295	Val	Pro	Tyr	Lys	Phe 300	Glu	Ser	Asp	Glu
Glu 305	Val	Tyr	Gln	Ser	Val 310	Asn	Gly	Phe	Leu	Asp 315	Asn	Ile	Ser	Ser	Lys 320
His	Ile	Val	Glu	Arg 325	Leu	Arg	Lys	Ile	Gly 330	Asp	Asn	Tyr	Asn	Gly 335	Tyr
Asn	Leu	Asp	Lys 340	Ile	Tyr	Ile	Val	Ser 345	Lys	Phe	Tyr	Glu	Ser	Val	Ser
Gln	Lys	Thr 355	Tyr	Arg	Asp	Trp	Glu 360	Thr	Ile	Asn	Thr	Ala 365	Leu	Glu	Ile
His 370	Tyr	Asn	Asn	Ile	Leu	Pro 375	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Asp	Lys
Val 385	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys 395	Ser	Ile	Thr	Glu	Ile 400
Asn	Glu	Leu	Val	Ser 405	Asn	Tyr	Lys	Leu	Cys 410	Ser	Asp	Asp	Asn	Ile	Lys 415
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn	Asn	Phe	Glu
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro 440	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
Leu 450	Lys	Ala	Ser	Glu	Leu	Lys 455	Asn	Val	Leu	Asp	Val 460	Ile	Met	Asn	Ala
Phe 465	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp 480
Asn	Asn	Phe	Tyr	Ala 485	Glu	Leu	Glu	Glu	Ile	Tyr 490	Asp	Glu	Ile	Tyr	Pro 495
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg 505	Asn	Tyr	Val	Thr	Gln	Lys	Pro 510
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu 520	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala 525
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu

-continued

530	535	540
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys 545 550 555 560		
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp 565 570 575		
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro Asn Lys Met Ile 580 585 590		
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro 595 600 605		
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser 610 615 620		
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe 625 630 635 640		
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp 645 650 655		
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu 660 665 670		
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys 675 680 685		
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile 690 695 700		
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His 705 710 715 720		
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile 725 730 735		
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser 740 745 750		
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg 755 760 765		
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val 770 775 780		
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe 785 790 795 800		
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys 805 810 815		
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr 820 825 830		
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn 835 840 845		
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr 850 855 860		
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu 865 870 875 880		
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val 885 890 895		
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys 900 905 910		
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys 915 920 925		
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val 930 935 940		
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala 945 950 955 960		

-continued

Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975

Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990

Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005

Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020

Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035

Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050

Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065

Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080

Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095

Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110

Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125

Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140

Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155

Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170

Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185

Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200

Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215

Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230

Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245

Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 79
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT S124T, K511I, N589H, K712V,
 K853R, H946W

<400> SEQUENCE: 79

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly

-continued

35	40	45
Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly		
50	55	60
Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser		
65	70	75
80		
Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp		
85	90	95
Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys		
100	105	110
Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Thr Ala Lys Leu Ile		
115	120	125
Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala		
130	135	140
Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe		
145	150	155
160		
Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser		
165	170	175
Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn		
180	185	190
Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys		
195	200	205
Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp		
210	215	220
Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr		
225	230	235
240		
Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys		
245	250	255
Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu		
260	265	270
Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys		
275	280	285
Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu		
290	295	300
Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys		
305	310	315
320		
His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr		
325	330	335
Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser		
340	345	350
Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile		
355	360	365
His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys		
370	375	380
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile		
385	390	395
400		
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys		
405	410	415
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu		
420	425	430
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu		
435	440	445
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala		
450	455	460

-continued

Phe	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp	
465					470					475					480	
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro	
				485					490					495		
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Ile	Pro	
			500					505					510			
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala	
		515					520					525				
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu	
	530					535					540					
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys	
545				550						555					560	
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp	
				565					570					575		
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	His	Lys	Met	Ile	
			580					585					590			
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro	
		595					600					605				
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser	
	610					615					620					
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe	
625					630					635					640	
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp	
			645						650					655		
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu	
			660					665					670			
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys	
		675					680					685				
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile	
	690					695					700					
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Val	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His	
705					710					715					720	
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile	
			725						730					735		
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser	
			740					745					750			
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg	
	755						760					765				
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val	
	770					775					780					
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe	
785				790						795					800	
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys	
				805					810					815		
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr	
			820					825					830			
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn	
			835					840					845			
Phe	Lys	Ala	Asn	Arg	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr	
	850					855					860					
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	
865					870					875					880	

-continued

Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val
 885 890 895

Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys
 900 905 910

Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925

Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940

Ile Trp Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960

Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975

Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990

Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005

Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val
 1010 1015 1020

Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035

Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050

Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065

Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080

Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095

Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110

Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125

Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140

Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155

Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170

Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185

Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200

Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215

Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230

Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245

Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 80

<211> LENGTH: 1263

<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT S124T, K511I, N589H, K786S, H946K, R1113F

<400> SEQUENCE: 80

```

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1             5             10             15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20             25             30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35             40             45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50             55             60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65             70             75             80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85             90             95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
100             105             110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Thr Ala Lys Leu Ile
115             120             125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
130             135             140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
145             150             155             160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
165             170             175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
180             185             190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
195             200             205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
210             215             220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
225             230             235             240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
245             250             255

Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
260             265             270

Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys
275             280             285

Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu
290             295             300

Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys
305             310             315             320

His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr
325             330             335

Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser
340             345             350

Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile
355             360             365

His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys
370             375             380

```


-continued

Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile	385	390	395	400
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys	405	410	415	
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu	420	425	430	
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu	435	440	445	
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala	450	455	460	
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp	465	470	475	480
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro	485	490	495	
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln Ile Pro	500	505	510	
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala	515	520	525	
Asp Gly Trp Ser Lys Ser Lys Glu Tyr Ser Asn Asn Ala Ile Ile Leu	530	535	540	
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys	545	550	555	560
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp	565	570	575	
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro His Lys Met Ile	580	585	590	
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro	595	600	605	
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser	610	615	620	
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe	625	630	635	640
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp	645	650	655	
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu	660	665	670	
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys	675	680	685	
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile	690	695	700	
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His	705	710	715	720
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile	725	730	735	
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser	740	745	750	
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg	755	760	765	
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val	770	775	780	
Arg Ser Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe	785	790	795	800
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys				

805										810					815				
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr				
			820				825						830						
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn				
			835				840						845						
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr				
			850				855						860						
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu				
			865				870						875	880					
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val				
			885				890						895						
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys				
			900				905						910						
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys				
			915				920						925						
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val				
			930				935						940						
Ile	Lys	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala				
			945				950						955	960					
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu				
			965				970						975						
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn				
			980				985						990						
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu				
			995				1000						1005						
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val					
			1010				1015						1020						
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr					
			1025				1030						1035						
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe					
			1040				1045						1050						
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe					
			1055				1060						1065						
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr					
			1070				1075						1080						
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys					
			1085				1090						1095						
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Phe					
			1100				1105						1110						
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile					
			1115				1120						1125						
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp					
			1130				1135						1140						
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile					
			1145				1150						1155						
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg					
			1160				1165						1170						
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile					
			1175				1180						1185						
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys					
			1190																

-continued

Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230
 Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245
 Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 81
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K511H, N589H, K853R, K1021L,
 D1181E, D1183E

<400> SEQUENCE: 81

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15
 Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30
 Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35 40 45
 Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50 55 60
 Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65 70 75 80
 Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85 90 95
 Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
 100 105 110
 Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Ser Ala Lys Leu Ile
 115 120 125
 Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
 130 135 140
 Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
 145 150 155 160
 Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
 165 170 175
 Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
 180 185 190
 Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
 195 200 205
 Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
 210 215 220
 Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
 225 230 235 240
 Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
 245 250 255
 Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
 260 265 270
 Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys
 275 280 285
 Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu
 290 295 300
 Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys

-continued

305	310	315	320
His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr	325	330	335
Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser	340	345	350
Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile	355	360	365
His Tyr Asn Asn Ile Leu Pro Gly Asn Gly Lys Ser Lys Ala Asp Lys	370	375	380
Val Lys Lys Ala Val Lys Asn Asp Leu Gln Lys Ser Ile Thr Glu Ile	385	390	395
Asn Glu Leu Val Ser Asn Tyr Lys Leu Cys Ser Asp Asp Asn Ile Lys	405	410	415
Ala Glu Thr Tyr Ile His Glu Ile Ser His Ile Leu Asn Asn Phe Glu	420	425	430
Ala Gln Glu Leu Lys Tyr Asn Pro Glu Ile His Leu Val Glu Ser Glu	435	440	445
Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala	450	455	460
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp	465	470	475
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro	485	490	495
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln His Pro	500	505	510
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala	515	520	525
Asp Gly Trp Ser Lys Ser Lys Glu Tyr Ser Asn Asn Ala Ile Ile Leu	530	535	540
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys	545	550	555
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp	565	570	575
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro His Lys Met Ile	580	585	590
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro	595	600	605
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser	610	615	620
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe	625	630	635
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp	645	650	655
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu	660	665	670
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys	675	680	685
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile	690	695	700
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His	705	710	715
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile	725	730	735

-continued

Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser
 740 745 750
 Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg
 755 760 765
 Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val
 770 775 780
 Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe
 785 790 795 800
 Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys
 805 810 815
 Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr
 820 825 830
 Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn
 835 840 845
 Phe Lys Ala Asn Arg Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr
 850 855 860
 Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu
 865 870 875 880
 Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val
 885 890 895
 Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys
 900 905 910
 Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys
 915 920 925
 Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val
 930 935 940
 Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala
 945 950 955 960
 Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu
 965 970 975
 Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn
 980 985 990
 Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu
 995 1000 1005
 Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Leu Asn Val
 1010 1015 1020
 Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr
 1025 1030 1035
 Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe
 1040 1045 1050
 Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe
 1055 1060 1065
 Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr
 1070 1075 1080
 Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095
 Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110
 Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125
 Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140

-continued

Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155
 Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170
 Asn Ser Leu Ser Glu Leu Glu Glu Arg Glu Tyr Asp Arg Leu Ile
 1175 1180 1185
 Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200
 Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215
 Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230
 Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245
 Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 82
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT K95L, S124T, K511I, N589H

<400> SEQUENCE: 82

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15
 Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30
 Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35 40 45
 Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50 55 60
 Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65 70 75 80
 Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Leu Asp
 85 90 95
 Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
 100 105 110
 Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Thr Ala Lys Leu Ile
 115 120 125
 Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
 130 135 140
 Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
 145 150 155 160
 Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
 165 170 175
 Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
 180 185 190
 Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
 195 200 205
 Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
 210 215 220
 Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
 225 230 235 240

Gly 245	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys
Gly 260	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu
Asn 275	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys
Ile 290	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu
Glu 305	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys
His 320	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
Asn 335	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
Gln 350	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
His 365	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
Val 380	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
Asn 400	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
Ala 415	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
Ala 430	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
Leu 445	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
Phe 460	His	Trp	Cys	Ser	Val	Phe	Met	Thr	Glu	Glu	Leu	Val	Asp	Lys	Asp
Asn 475	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro
Val 490	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg	Asn	Tyr	Val	Thr	Gln	Ile	Pro
Tyr 505	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
Asp 520	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
Met 535	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
Pro 550	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
Tyr 565	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	His	Lys	Met	Ile
Pro 580	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
Ser 595	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
Ser 610	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
Lys 625	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
Phe 640	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu

-continued

660	665	670
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys 675 680 685		
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile 690 695 700		
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His 705 710 715 720		
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile 725 730 735		
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser 740 745 750		
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg 755 760 765		
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val 770 775 780		
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe 785 790 795 800		
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys 805 810 815		
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr 820 825 830		
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn 835 840 845		
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr 850 855 860		
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu 865 870 875 880		
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val 885 890 895		
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys 900 905 910		
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys 915 920 925		
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val 930 935 940		
Ile His Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala 945 950 955 960		
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu 965 970 975		
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn 980 985 990		
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu 995 1000 1005		
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val 1010 1015 1020		
Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr 1025 1030 1035		
Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe 1040 1045 1050		
Lys Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe 1055 1060 1065		
Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr 1070 1075 1080		

-continued

Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys
 1085 1090 1095
 Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg
 1100 1105 1110
 Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile
 1115 1120 1125
 Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp
 1130 1135 1140
 Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile
 1145 1150 1155
 Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg
 1160 1165 1170
 Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile
 1175 1180 1185
 Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys
 1190 1195 1200
 Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215
 Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230
 Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245
 Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 83
 <211> LENGTH: 1263
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 VARIANT S124T, K511I, N589H, K712L,
 K786S, K1021V

<400> SEQUENCE: 83

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1 5 10 15
 Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20 25 30
 Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35 40 45
 Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50 55 60
 Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65 70 75 80
 Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85 90 95
 Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
 100 105 110
 Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Thr Ala Lys Leu Ile
 115 120 125
 Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Tyr Ser Ala
 130 135 140
 Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
 145 150 155 160
 Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser

-continued

165								170				175			
Ala	Asp	Asp	Ile 180	Ser	Ser	Ser	Ser	Cys 185	His	Arg	Ile	Val	Asn 190	Asp	Asn
Ala	Glu	Ile	Phe 195	Phe	Ser	Asn	Ala 200	Leu	Val	Tyr	Arg	Arg 205	Ile	Val	Lys
Ser	Leu	Ser	Asn	Asp	Asp	Ile 215	Asn	Lys	Ile	Ser	Gly 220	Asp	Met	Lys	Asp
Ser	Leu	Lys	Glu	Met	Ser 230	Leu	Glu	Glu	Ile	Tyr 235	Ser	Tyr	Glu	Lys	Tyr 240
Gly	Glu	Phe	Ile	Thr 245	Gln	Glu	Gly	Ile	Ser 250	Phe	Tyr	Asn	Asp	Ile	Cys 255
Gly	Lys	Val	Asn 260	Ser	Phe	Met	Asn	Leu 265	Tyr	Cys	Gln	Lys	Asn 270	Lys	Glu
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln 280	Lys	Leu	His	Lys	Gln 285	Ile	Leu	Cys
Ile	Ala	Asp	Thr	Ser	Tyr	Glu 295	Val	Pro	Tyr	Lys	Phe 300	Glu	Ser	Asp	Glu
Glu	Val	Tyr	Gln	Ser	Val 310	Asn	Gly	Phe	Leu	Asp 315	Asn	Ile	Ser	Ser	Lys 320
His	Ile	Val	Glu	Arg 325	Leu	Arg	Lys	Ile	Gly 330	Asp	Asn	Tyr	Asn	Gly	Tyr 335
Asn	Leu	Asp	Lys 340	Ile	Tyr	Ile	Val	Ser 345	Lys	Phe	Tyr	Glu	Ser	Val	Ser
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu 360	Thr	Ile	Asn	Thr	Ala 365	Leu	Glu	Ile
His	Tyr	Asn	Asn	Ile	Leu	Pro 375	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Asp	Lys
Val	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys 395	Ser	Ile	Thr	Glu	Ile 400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys 410	Ser	Asp	Asp	Asn	Ile	Lys
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn 430	Asn	Phe	Glu
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro 440	Glu	Ile	His	Leu	Val 445	Glu	Ser	Glu
Leu	Lys	Ala	Ser	Glu	Leu	Lys 455	Asn	Val	Leu	Asp	Val 460	Ile	Met	Asn	Ala
Phe	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu	Leu	Val 475	Asp	Lys	Asp 480
Asn	Asn	Phe	Tyr	Ala	Glu	Leu	Glu	Glu	Ile	Tyr	Asp	Glu	Ile	Tyr	Pro 495
Val	Ile	Ser	Leu	Tyr	Asn	Leu	Val	Arg 505	Asn	Tyr	Val	Thr	Gln 510	Ile	Pro
Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu 520	Asn	Phe	Gly	Ile	Pro 525	Thr	Leu	Ala
Asp	Gly	Trp	Ser	Lys	Ser	Lys 535	Glu	Tyr	Ser	Asn	Asn	Ala 540	Ile	Ile	Leu
Met	Arg	Asp	Asn	Leu	Tyr 550	Tyr	Leu	Gly	Ile	Phe	Asn	Ala 555	Lys	Asn	Lys 560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp 575
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu 585	Pro	Gly	Pro	His	Lys 590	Met	Ile

Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro				
595		600														605			
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser				
610		615														620			
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe				
625		630														635		640	
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp				
		645														650		655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu				
		660														665		670	
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys				
675		680														685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile				
690		695														700			
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Leu	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His				
705		710														715		720	
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile				
		725														730		735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser				
		740														745		750	
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg				
755		760														765			
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val				
770		775														780			
Arg	Ser	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe				
785		790														795		800	
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys				
		805														810		815	
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr				
		820														825		830	
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn				
835		840														845			
Phe	Lys	Ala	Asn	Lys	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr				
850		855														860			
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu				
865		870														875		880	
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val				
		885														890		895	
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys				
		900														905		910	
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys				
915		920														925			
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val				
930		935														940			
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala				
945		950														955		960	
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu				
		965														970		975	
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn				
		980														985		990	
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu				
995		1000														1005			

Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Val	Asn	Val
1010						1015					1020			
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr
1025						1030					1035			
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Lys	Phe
1040						1045					1050			
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe
1055						1060					1065			
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr
1070						1075					1080			
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys
1085						1090					1095			
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg
1100						1105					1110			
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile
1115						1120					1125			
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp
1130						1135					1140			
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile
1145						1150					1155			
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg
1160						1165					1170			
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile
1175						1180					1185			
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys
1190						1195					1200			
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr
1205						1210					1215			
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu
1220						1225					1230			
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile
1235						1240					1245			
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu
1250						1255					1260			
<210> SEQ ID NO 84														
<211> LENGTH: 1263														
<212> TYPE: PRT														
<213> ORGANISM: Artificial Sequence														
<220> FEATURE:														
<223> OTHER INFORMATION: MAD70 VARIANT S124T, K511H, N589H, K853R, H946K, K1054Y														
<400> SEQUENCE: 84														
Met	Asn	Asn	Gly	Thr										

Thr	Leu	Ile	Lys	Glu	Gln	Thr	Glu	Tyr	Arg	Lys	Ala	Ile	His	Lys	Lys
			100				105						110		
Phe	Ala	Asn	Asp	Asp	Arg	Phe	Lys	Asn	Met	Phe	Thr	Ala	Lys	Leu	Ile
			115				120					125			
Ser	Asp	Ile	Leu	Pro	Glu	Phe	Val	Ile	His	Asn	Asn	Asn	Tyr	Ser	Ala
			130			135				140					
Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe
			145		150					155					160
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser
				165					170					175	
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn
			180					185					190		
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys
			195				200					205			
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp
			210			215					220				
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr
			225		230					235					240
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys
				245					250					255	
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu
			260					265					270		
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys
			275			280						285			
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu
			290			295					300				
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys
			305		310					315					320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
				325					330					335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
			340					345					350		
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
			355			360						365			
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
			370		375						380				
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
					390					395					400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
			405					410					415		
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
			420					425				430			
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
			435			440						445			
Leu	Lys	Ala	Ser	Glu	Leu	Lys	Asn	Val	Leu	Asp	Val	Ile	Met	Asn	Ala
			450		455			</							

-continued

Tyr	Ser	Thr	Lys	Lys	Ile	Lys	Leu	Asn	Phe	Gly	Ile	Pro	Thr	Leu	Ala
		515					520					525			
Asp	Gly	Trp	Ser	Lys	Ser	Lys	Glu	Tyr	Ser	Asn	Asn	Ala	Ile	Ile	Leu
	530					535					540				
Met	Arg	Asp	Asn	Leu	Tyr	Tyr	Leu	Gly	Ile	Phe	Asn	Ala	Lys	Asn	Lys
	545				550					555					560
Pro	Asp	Lys	Lys	Ile	Ile	Glu	Gly	Asn	Thr	Ser	Glu	Asn	Lys	Gly	Asp
				565					570					575	
Tyr	Lys	Lys	Met	Ile	Tyr	Asn	Leu	Leu	Pro	Gly	Pro	His	Lys	Met	Ile
			580					585					590		
Pro	Lys	Val	Phe	Leu	Ser	Ser	Lys	Thr	Gly	Val	Glu	Thr	Tyr	Lys	Pro
		595					600					605			
Ser	Ala	Tyr	Ile	Leu	Glu	Gly	Tyr	Lys	Gln	Asn	Lys	His	Ile	Lys	Ser
	610					615					620				
Ser	Lys	Asp	Phe	Asp	Ile	Thr	Phe	Cys	His	Asp	Leu	Ile	Asp	Tyr	Phe
	625				630					635					640
Lys	Asn	Cys	Ile	Ala	Ile	His	Pro	Glu	Trp	Lys	Asn	Phe	Gly	Phe	Asp
			645						650					655	
Phe	Ser	Asp	Thr	Ser	Thr	Tyr	Glu	Asp	Ile	Ser	Gly	Phe	Tyr	Arg	Glu
			660					665					670		
Val	Glu	Leu	Gln	Gly	Tyr	Lys	Ile	Asp	Trp	Thr	Tyr	Ile	Ser	Glu	Lys
		675					680					685			
Asp	Ile	Asp	Leu	Leu	Gln	Glu	Lys	Gly	Gln	Leu	Tyr	Leu	Phe	Gln	Ile
	690					695					700				
Tyr	Asn	Lys	Asp	Phe	Ser	Lys	Lys	Ser	Thr	Gly	Asn	Asp	Asn	Leu	His
	705				710					715					720
Thr	Met	Tyr	Leu	Lys	Asn	Leu	Phe	Ser	Glu	Glu	Asn	Leu	Lys	Asp	Ile
				725					730					735	
Val	Leu	Lys	Leu	Asn	Gly	Glu	Ala	Glu	Ile	Phe	Phe	Arg	Lys	Ser	Ser
			740					745					750		
Ile	Lys	Asn	Pro	Ile	Ile	His	Lys	Lys	Gly	Ser	Ile	Leu	Val	Asn	Arg
		755					760					765			
Thr	Tyr	Glu	Ala	Glu	Glu	Lys	Asp	Gln	Phe	Gly	Asn	Ile	Gln	Ile	Val
	770					775					780				
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Tyr	Phe
	785				790					795					800
Asn	Asp	Lys	Ser	Asp	Lys	Glu	Leu	Ser	Asp	Glu	Ala	Ala	Lys	Leu	Lys
				805					810					815	
Asn	Val	Val	Gly	His	His	Glu	Ala	Ala	Thr	Asn	Ile	Val	Lys	Asp	Tyr
			820					825					830		
Arg	Tyr	Thr	Tyr	Asp	Lys	Tyr	Phe	Leu	His	Met	Pro	Ile	Thr	Ile	Asn
			835				840						845		
Phe	Lys	Ala	Asn	Arg	Thr	Gly	Phe	Ile	Asn	Asp	Arg	Ile	Leu	Gln	Tyr
	850					855					860				
Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu
	865				870					875					880
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val
				885					890					895	
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys
			900					905					910		
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys
		915					920					925			
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val

-continued

930	935	940
Ile Lys Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala		
945	950	955 960
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu		
	965	970 975
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn		
	980	985 990
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu		
	995	1000 1005
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val		
	1010	1015 1020
Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr		
	1025	1030 1035
Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Lys Phe		
	1040	1045 1050
Tyr Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe		
	1055	1060 1065
Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr		
	1070	1075 1080
Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys		
	1085	1090 1095
Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg		
	1100	1105 1110
Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile		
	1115	1120 1125
Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp		
	1130	1135 1140
Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile		
	1145	1150 1155
Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg		
	1160	1165 1170
Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile		
	1175	1180 1185
Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys		
	1190	1195 1200
Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr		
	1205	1210 1215
Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu		
	1220	1225 1230
Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile		
	1235	1240 1245
Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu		
	1250	1255 1260

<210> SEQ ID NO 85

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MAD70 VARIANT K95L, S124T, N589H, K853R, K1052Q

<400> SEQUENCE: 85

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
1 5 10 15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln

-continued

20					25					30					
Gln	Phe	Ile	Val	Lys	Asn	Gly	Ile	Ile	Lys	Glu	Asp	Glu	Leu	Arg	Gly
		35					40					45			
Glu	Asn	Arg	Gln	Ile	Leu	Lys	Asp	Ile	Met	Asp	Asp	Tyr	Tyr	Arg	Gly
	50					55					60				
Phe	Ile	Ser	Glu	Thr	Leu	Ser	Ser	Ile	Asp	Asp	Ile	Asp	Trp	Thr	Ser
65					70					75					80
Leu	Phe	Glu	Lys	Met	Glu	Ile	Gln	Leu	Lys	Asn	Gly	Asp	Asn	Leu	Asp
			85						90					95	
Thr	Leu	Ile	Lys	Glu	Gln	Thr	Glu	Tyr	Arg	Lys	Ala	Ile	His	Lys	Lys
			100					105					110		
Phe	Ala	Asn	Asp	Asp	Arg	Phe	Lys	Asn	Met	Phe	Thr	Ala	Lys	Leu	Ile
		115					120					125			
Ser	Asp	Ile	Leu	Pro	Glu	Phe	Val	Ile	His	Asn	Asn	Asn	Tyr	Ser	Ala
	130					135					140				
Ser	Glu	Lys	Glu	Glu	Lys	Thr	Gln	Val	Ile	Lys	Leu	Phe	Ser	Arg	Phe
145					150					155					160
Ala	Thr	Ser	Phe	Lys	Asp	Tyr	Phe	Lys	Asn	Arg	Ala	Asn	Cys	Phe	Ser
			165						170					175	
Ala	Asp	Asp	Ile	Ser	Ser	Ser	Ser	Cys	His	Arg	Ile	Val	Asn	Asp	Asn
		180						185					190		
Ala	Glu	Ile	Phe	Phe	Ser	Asn	Ala	Leu	Val	Tyr	Arg	Arg	Ile	Val	Lys
		195					200					205			
Ser	Leu	Ser	Asn	Asp	Asp	Ile	Asn	Lys	Ile	Ser	Gly	Asp	Met	Lys	Asp
	210					215					220				
Ser	Leu	Lys	Glu	Met	Ser	Leu	Glu	Glu	Ile	Tyr	Ser	Tyr	Glu	Lys	Tyr
225					230					235					240
Gly	Glu	Phe	Ile	Thr	Gln	Glu	Gly	Ile	Ser	Phe	Tyr	Asn	Asp	Ile	Cys
			245					250						255	
Gly	Lys	Val	Asn	Ser	Phe	Met	Asn	Leu	Tyr	Cys	Gln	Lys	Asn	Lys	Glu
		260						265					270		
Asn	Lys	Asn	Leu	Tyr	Lys	Leu	Gln	Lys	Leu	His	Lys	Gln	Ile	Leu	Cys
		275					280					285			
Ile	Ala	Asp	Thr	Ser	Tyr	Glu	Val	Pro	Tyr	Lys	Phe	Glu	Ser	Asp	Glu
	290					295					300				
Glu	Val	Tyr	Gln	Ser	Val	Asn	Gly	Phe	Leu	Asp	Asn	Ile	Ser	Ser	Lys
305					310					315					320
His	Ile	Val	Glu	Arg	Leu	Arg	Lys	Ile	Gly	Asp	Asn	Tyr	Asn	Gly	Tyr
			325					330						335	
Asn	Leu	Asp	Lys	Ile	Tyr	Ile	Val	Ser	Lys	Phe	Tyr	Glu	Ser	Val	Ser
		340						345					350		
Gln	Lys	Thr	Tyr	Arg	Asp	Trp	Glu	Thr	Ile	Asn	Thr	Ala	Leu	Glu	Ile
		355				360						365			
His	Tyr	Asn	Asn	Ile	Leu	Pro	Gly	Asn	Gly	Lys	Ser	Lys	Ala	Asp	Lys
	370				375					380					
Val	Lys	Lys	Ala	Val	Lys	Asn	Asp	Leu	Gln	Lys	Ser	Ile	Thr	Glu	Ile
385					390					395					400
Asn	Glu	Leu	Val	Ser	Asn	Tyr	Lys	Leu	Cys	Ser	Asp	Asp	Asn	Ile	Lys
		405						410					415		
Ala	Glu	Thr	Tyr	Ile	His	Glu	Ile	Ser	His	Ile	Leu	Asn	Asn	Phe	Glu
		420						425					430		
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro	Glu	Ile	His	Leu	Val	Glu	Ser	Glu
	435					440						445			

-continued

Leu Lys Ala Ser Glu Leu Lys Asn Val Leu Asp Val Ile Met Asn Ala	
450	455 460
Phe His Trp Cys Ser Val Phe Met Thr Glu Glu Leu Val Asp Lys Asp	
465	470 475 480
Asn Asn Phe Tyr Ala Glu Leu Glu Glu Ile Tyr Asp Glu Ile Tyr Pro	
	485 490 495
Val Ile Ser Leu Tyr Asn Leu Val Arg Asn Tyr Val Thr Gln His Pro	
	500 505 510
Tyr Ser Thr Lys Lys Ile Lys Leu Asn Phe Gly Ile Pro Thr Leu Ala	
	515 520 525
Asp Gly Trp Ser Lys Ser Lys Glu Tyr Ser Asn Asn Ala Ile Ile Leu	
	530 535 540
Met Arg Asp Asn Leu Tyr Tyr Leu Gly Ile Phe Asn Ala Lys Asn Lys	
	545 550 555 560
Pro Asp Lys Lys Ile Ile Glu Gly Asn Thr Ser Glu Asn Lys Gly Asp	
	565 570 575
Tyr Lys Lys Met Ile Tyr Asn Leu Leu Pro Gly Pro His Lys Met Ile	
	580 585 590
Pro Lys Val Phe Leu Ser Ser Lys Thr Gly Val Glu Thr Tyr Lys Pro	
	595 600 605
Ser Ala Tyr Ile Leu Glu Gly Tyr Lys Gln Asn Lys His Ile Lys Ser	
	610 615 620
Ser Lys Asp Phe Asp Ile Thr Phe Cys His Asp Leu Ile Asp Tyr Phe	
	625 630 635 640
Lys Asn Cys Ile Ala Ile His Pro Glu Trp Lys Asn Phe Gly Phe Asp	
	645 650 655
Phe Ser Asp Thr Ser Thr Tyr Glu Asp Ile Ser Gly Phe Tyr Arg Glu	
	660 665 670
Val Glu Leu Gln Gly Tyr Lys Ile Asp Trp Thr Tyr Ile Ser Glu Lys	
	675 680 685
Asp Ile Asp Leu Leu Gln Glu Lys Gly Gln Leu Tyr Leu Phe Gln Ile	
	690 695 700
Tyr Asn Lys Asp Phe Ser Lys Lys Ser Thr Gly Asn Asp Asn Leu His	
	705 710 715 720
Thr Met Tyr Leu Lys Asn Leu Phe Ser Glu Glu Asn Leu Lys Asp Ile	
	725 730 735
Val Leu Lys Leu Asn Gly Glu Ala Glu Ile Phe Phe Arg Lys Ser Ser	
	740 745 750
Ile Lys Asn Pro Ile Ile His Lys Lys Gly Ser Ile Leu Val Asn Arg	
	755 760 765
Thr Tyr Glu Ala Glu Glu Lys Asp Gln Phe Gly Asn Ile Gln Ile Val	
	770 775 780
Arg Lys Asn Ile Pro Glu Asn Ile Tyr Gln Glu Leu Tyr Lys Tyr Phe	
	785 790 795 800
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys	
	805 810 815
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr	
	820 825 830
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn	
	835 840 845
Phe Lys Ala Asn Arg Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr	
	850 855 860

-continued

Ile	Ala	Lys	Glu	Lys	Asp	Leu	His	Val	Ile	Gly	Ile	Asp	Arg	Gly	Glu	865	870	875	880
Arg	Asn	Leu	Ile	Tyr	Val	Ser	Val	Ile	Asp	Thr	Cys	Gly	Asn	Ile	Val	885	890	895	
Glu	Gln	Lys	Ser	Phe	Asn	Ile	Val	Asn	Gly	Tyr	Asp	Tyr	Gln	Ile	Lys	900	905	910	
Leu	Lys	Gln	Gln	Glu	Gly	Ala	Arg	Gln	Ile	Ala	Arg	Lys	Glu	Trp	Lys	915	920	925	
Glu	Ile	Gly	Lys	Ile	Lys	Glu	Ile	Lys	Glu	Gly	Tyr	Leu	Ser	Leu	Val	930	935	940	
Ile	His	Glu	Ile	Ser	Lys	Met	Val	Ile	Lys	Tyr	Asn	Ala	Ile	Ile	Ala	945	950	955	960
Met	Glu	Asp	Leu	Ser	Tyr	Gly	Phe	Lys	Lys	Gly	Arg	Phe	Lys	Val	Glu	965	970	975	
Arg	Gln	Val	Tyr	Gln	Lys	Phe	Glu	Thr	Met	Leu	Ile	Asn	Lys	Leu	Asn	980	985	990	
Tyr	Leu	Val	Phe	Lys	Asp	Ile	Ser	Ile	Thr	Glu	Asn	Gly	Gly	Leu	Leu	995	1000	1005	
Lys	Gly	Tyr	Gln	Leu	Thr	Tyr	Ile	Pro	Asp	Lys	Leu	Lys	Asn	Val		1010	1015	1020	
Gly	His	Gln	Cys	Gly	Cys	Ile	Phe	Tyr	Val	Pro	Ala	Ala	Tyr	Thr		1025	1030	1035	
Ser	Lys	Ile	Asp	Pro	Thr	Thr	Gly	Phe	Val	Asn	Ile	Phe	Gln	Phe		1040	1045	1050	
Lys	Asp	Leu	Thr	Val	Asp	Ala	Lys	Arg	Glu	Phe	Ile	Lys	Lys	Phe		1055	1060	1065	
Asp	Ser	Ile	Arg	Tyr	Asp	Ser	Glu	Lys	Asn	Leu	Phe	Cys	Phe	Thr		1070	1075	1080	
Phe	Asp	Tyr	Asn	Asn	Phe	Ile	Thr	Gln	Asn	Thr	Val	Met	Ser	Lys		1085	1090	1095	
Ser	Ser	Trp	Ser	Val	Tyr	Thr	Tyr	Gly	Val	Arg	Ile	Lys	Arg	Arg		1100	1105	1110	
Phe	Val	Asn	Gly	Arg	Phe	Ser	Asn	Glu	Ser	Asp	Thr	Ile	Asp	Ile		1115	1120	1125	
Thr	Lys	Asp	Met	Glu	Lys	Thr	Leu	Glu	Met	Thr	Asp	Ile	Asn	Trp		1130	1135	1140	
Arg	Asp	Gly	His	Asp	Leu	Arg	Gln	Asp	Ile	Ile	Asp	Tyr	Glu	Ile		1145	1150	1155	
Val	Gln	His	Ile	Phe	Glu	Ile	Phe	Arg	Leu	Thr	Val	Gln	Met	Arg		1160	1165	1170	
Asn	Ser	Leu	Ser	Glu	Leu	Glu	Asp	Arg	Asp	Tyr	Asp	Arg	Leu	Ile		1175	1180	1185	
Ser	Pro	Val	Leu	Asn	Glu	Asn	Asn	Ile	Phe	Tyr	Asp	Ser	Ala	Lys		1190	1195	1200	
Ala	Gly	Asp	Ala	Leu	Pro	Lys	Asp	Ala	Asp	Ala	Asn	Gly	Ala	Tyr		1205	1210	1215	
Cys	Ile	Ala	Leu	Lys	Gly	Leu	Tyr	Glu	Ile	Lys	Gln	Ile	Thr	Glu		1220	1225	1230	
Asn	Trp	Lys	Glu	Asp	Gly	Lys	Phe	Ser	Arg	Asp	Lys	Leu	Lys	Ile		1235	1240	1245	
Ser	Asn	Lys	Asp	Trp	Phe	Asp	Phe	Ile	Gln	Asn	Lys	Arg	Tyr	Leu		1250	1255	1260	

-continued

```

<210> SEQ ID NO 86
<211> LENGTH: 1263
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MAD70 VARIANT S124T, K511I, N589H, K712L,
      H946T, K1052Q, K1054N

<400> SEQUENCE: 86

Met Asn Asn Gly Thr Asn Asn Phe Gln Asn Phe Ile Gly Ile Ser Ser
 1             5             10             15

Leu Gln Lys Thr Leu Arg Asn Ala Leu Ile Pro Thr Glu Thr Thr Gln
 20             25             30

Gln Phe Ile Val Lys Asn Gly Ile Ile Lys Glu Asp Glu Leu Arg Gly
 35             40             45

Glu Asn Arg Gln Ile Leu Lys Asp Ile Met Asp Asp Tyr Tyr Arg Gly
 50             55             60

Phe Ile Ser Glu Thr Leu Ser Ser Ile Asp Asp Ile Asp Trp Thr Ser
 65             70             75             80

Leu Phe Glu Lys Met Glu Ile Gln Leu Lys Asn Gly Asp Asn Lys Asp
 85             90             95

Thr Leu Ile Lys Glu Gln Thr Glu Tyr Arg Lys Ala Ile His Lys Lys
100            105            110

Phe Ala Asn Asp Asp Arg Phe Lys Asn Met Phe Thr Ala Lys Leu Ile
115            120            125

Ser Asp Ile Leu Pro Glu Phe Val Ile His Asn Asn Asn Tyr Ser Ala
130            135            140

Ser Glu Lys Glu Glu Lys Thr Gln Val Ile Lys Leu Phe Ser Arg Phe
145            150            155            160

Ala Thr Ser Phe Lys Asp Tyr Phe Lys Asn Arg Ala Asn Cys Phe Ser
165            170            175

Ala Asp Asp Ile Ser Ser Ser Ser Cys His Arg Ile Val Asn Asp Asn
180            185            190

Ala Glu Ile Phe Phe Ser Asn Ala Leu Val Tyr Arg Arg Ile Val Lys
195            200            205

Ser Leu Ser Asn Asp Asp Ile Asn Lys Ile Ser Gly Asp Met Lys Asp
210            215            220

Ser Leu Lys Glu Met Ser Leu Glu Glu Ile Tyr Ser Tyr Glu Lys Tyr
225            230            235            240

Gly Glu Phe Ile Thr Gln Glu Gly Ile Ser Phe Tyr Asn Asp Ile Cys
245            250            255

Gly Lys Val Asn Ser Phe Met Asn Leu Tyr Cys Gln Lys Asn Lys Glu
260            265            270

Asn Lys Asn Leu Tyr Lys Leu Gln Lys Leu His Lys Gln Ile Leu Cys
275            280            285

Ile Ala Asp Thr Ser Tyr Glu Val Pro Tyr Lys Phe Glu Ser Asp Glu
290            295            300

Glu Val Tyr Gln Ser Val Asn Gly Phe Leu Asp Asn Ile Ser Ser Lys
305            310            315            320

His Ile Val Glu Arg Leu Arg Lys Ile Gly Asp Asn Tyr Asn Gly Tyr
325            330            335

Asn Leu Asp Lys Ile Tyr Ile Val Ser Lys Phe Tyr Glu Ser Val Ser
340            345            350

Gln Lys Thr Tyr Arg Asp Trp Glu Thr Ile Asn Thr Ala Leu Glu Ile
355            360            365

```

His 370	Asn	Asn	Ile	Leu	Pro 375	Gly	Asn	Gly	Lys	Ser 380	Lys	Ala	Asp	Lys
Val 385	Lys	Lys	Ala	Val	Lys 390	Asn	Asp	Leu	Gln	Lys 395	Ser	Ile	Thr	Ile 400
Asn	Glu	Leu	Val	Ser 405	Asn	Tyr	Lys	Leu	Cys 410	Ser	Asp	Asp	Asn	Lys 415
Ala	Glu	Thr	Tyr 420	Ile	His	Glu	Ile	Ser 425	His	Ile	Leu	Asn	Asn	Glu 430
Ala	Gln	Glu	Leu	Lys	Tyr	Asn	Pro 440	Glu	Ile	His	Leu	Val	Glu	Glu 445
Leu	Lys 450	Ala	Ser	Glu	Leu	Lys 455	Asn	Val	Leu	Asp	Val 460	Ile	Met	Ala 465
Phe 465	His	Trp	Cys	Ser	Val 470	Phe	Met	Thr	Glu	Glu 475	Leu	Val	Asp	Asp 480
Asn	Asn	Phe	Tyr 485	Ala	Glu	Leu	Glu	Glu	Ile 490	Tyr	Asp	Glu	Ile	Pro 495
Val	Ile	Ser	Leu 500	Tyr	Asn	Leu	Val	Arg 505	Asn	Tyr	Val	Thr 510	Gln	Pro 515
Tyr	Ser	Thr 515	Lys	Lys	Ile	Lys	Leu 520	Asn	Phe	Gly	Ile	Pro 525	Thr	Ala 530
Asp 530	Gly	Trp	Ser	Lys	Ser	Lys 535	Glu	Tyr	Ser	Asn 540	Asn	Ala	Ile	Leu 545
Met 545	Arg	Asp	Asn	Leu	Tyr 550	Tyr	Leu	Gly	Ile	Phe 555	Asn	Ala	Lys	Lys 560
Pro	Asp	Lys	Lys 565	Ile	Ile	Glu	Gly	Asn	Thr 570	Ser	Glu	Asn	Lys	Asp 575
Tyr	Lys	Lys	Met 580	Ile	Tyr	Asn	Leu	Leu 585	Pro	Gly	Pro	His 590	Lys	Ile 595
Pro	Lys 595	Val	Phe	Leu	Ser	Ser	Lys 600	Thr	Gly	Val	Glu	Thr 605	Tyr	Pro 610
Ser 610	Ala	Tyr	Ile	Leu	Glu	Gly 615	Tyr	Lys	Gln	Asn 620	Lys	His	Ile	Ser 625
Ser 625	Lys	Asp	Phe	Asp	Ile 630	Thr	Phe	Cys	His	Asp 635	Leu	Ile	Asp	Phe 640
Lys	Asn	Cys	Ile 645	Ala	Ile	His	Pro	Glu	Trp 650	Lys	Asn	Phe	Gly	Asp 655
Phe	Ser	Asp	Thr 660	Ser	Thr	Tyr	Glu	Asp 665	Ile	Ser	Gly	Phe 670	Tyr	Glu 675
Val	Glu	Leu	Gln 675	Gly	Tyr	Lys	Ile 680	Asp	Trp	Thr	Tyr 685	Ile	Ser	Lys 690
Asp 690	Ile	Asp	Leu	Leu	Gln 695	Glu	Lys	Gly	Gln	Leu 700	Tyr	Leu	Phe	Ile 705
Tyr 705	Asn	Lys	Asp	Phe	Ser 710	Lys	Leu	Ser	Thr	Gly 715	Asn	Asp	Asn	His 720
Thr	Met	Tyr	Leu 725	Lys	Asn	Leu	Phe	Ser	Glu 730	Glu	Asn	Leu	Lys	Ile 735
Val	Leu	Lys	Leu 740	Asn	Gly	Glu	Ala	Glu 745	Ile	Phe	Phe	Arg 750	Lys	Ser 755
Ile	Lys 755	Asn	Pro	Ile	Ile	His	Lys 760	Lys	Gly	Ser	Ile	Leu 765	Val	Arg 770
Thr 770	Tyr	Glu	Ala	Glu	Glu 775	Lys	Asp	Gln	Phe	Gly 780	Asn	Ile	Gln	Val 785
Arg	Lys	Asn	Ile	Pro	Glu	Asn	Ile	Tyr	Gln	Glu	Leu	Tyr	Lys	Pro 790

-continued

785	790	795	800
Asn Asp Lys Ser Asp Lys Glu Leu Ser Asp Glu Ala Ala Lys Leu Lys	805	810	815
Asn Val Val Gly His His Glu Ala Ala Thr Asn Ile Val Lys Asp Tyr	820	825	830
Arg Tyr Thr Tyr Asp Lys Tyr Phe Leu His Met Pro Ile Thr Ile Asn	835	840	845
Phe Lys Ala Asn Lys Thr Gly Phe Ile Asn Asp Arg Ile Leu Gln Tyr	850	855	860
Ile Ala Lys Glu Lys Asp Leu His Val Ile Gly Ile Asp Arg Gly Glu	865	870	875
Arg Asn Leu Ile Tyr Val Ser Val Ile Asp Thr Cys Gly Asn Ile Val	885	890	895
Glu Gln Lys Ser Phe Asn Ile Val Asn Gly Tyr Asp Tyr Gln Ile Lys	900	905	910
Leu Lys Gln Gln Glu Gly Ala Arg Gln Ile Ala Arg Lys Glu Trp Lys	915	920	925
Glu Ile Gly Lys Ile Lys Glu Ile Lys Glu Gly Tyr Leu Ser Leu Val	930	935	940
Ile Thr Glu Ile Ser Lys Met Val Ile Lys Tyr Asn Ala Ile Ile Ala	945	950	955
Met Glu Asp Leu Ser Tyr Gly Phe Lys Lys Gly Arg Phe Lys Val Glu	965	970	975
Arg Gln Val Tyr Gln Lys Phe Glu Thr Met Leu Ile Asn Lys Leu Asn	980	985	990
Tyr Leu Val Phe Lys Asp Ile Ser Ile Thr Glu Asn Gly Gly Leu Leu	995	1000	1005
Lys Gly Tyr Gln Leu Thr Tyr Ile Pro Asp Lys Leu Lys Asn Val	1010	1015	1020
Gly His Gln Cys Gly Cys Ile Phe Tyr Val Pro Ala Ala Tyr Thr	1025	1030	1035
Ser Lys Ile Asp Pro Thr Thr Gly Phe Val Asn Ile Phe Gln Phe	1040	1045	1050
Asn Asp Leu Thr Val Asp Ala Lys Arg Glu Phe Ile Lys Lys Phe	1055	1060	1065
Asp Ser Ile Arg Tyr Asp Ser Glu Lys Asn Leu Phe Cys Phe Thr	1070	1075	1080
Phe Asp Tyr Asn Asn Phe Ile Thr Gln Asn Thr Val Met Ser Lys	1085	1090	1095
Ser Ser Trp Ser Val Tyr Thr Tyr Gly Val Arg Ile Lys Arg Arg	1100	1105	1110
Phe Val Asn Gly Arg Phe Ser Asn Glu Ser Asp Thr Ile Asp Ile	1115	1120	1125
Thr Lys Asp Met Glu Lys Thr Leu Glu Met Thr Asp Ile Asn Trp	1130	1135	1140
Arg Asp Gly His Asp Leu Arg Gln Asp Ile Ile Asp Tyr Glu Ile	1145	1150	1155
Val Gln His Ile Phe Glu Ile Phe Arg Leu Thr Val Gln Met Arg	1160	1165	1170
Asn Ser Leu Ser Glu Leu Glu Asp Arg Asp Tyr Asp Arg Leu Ile	1175	1180	1185
Ser Pro Val Leu Asn Glu Asn Asn Ile Phe Tyr Asp Ser Ala Lys	1190	1195	1200

-continued

Ala Gly Asp Ala Leu Pro Lys Asp Ala Asp Ala Asn Gly Ala Tyr
 1205 1210 1215

Cys Ile Ala Leu Lys Gly Leu Tyr Glu Ile Lys Gln Ile Thr Glu
 1220 1225 1230

Asn Trp Lys Glu Asp Gly Lys Phe Ser Arg Asp Lys Leu Lys Ile
 1235 1240 1245

Ser Asn Lys Asp Trp Phe Asp Phe Ile Gln Asn Lys Arg Tyr Leu
 1250 1255 1260

<210> SEQ ID NO 87
 <211> LENGTH: 8059
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EE0026 VECTOR SEQ

<400> SEQUENCE: 87

```

gctgtttgct cctctacaaa acagacctta aaaccctaaa ggcttaagta gcaccctcgc      60
aagctcgggt gcggccgcaa tcgggcaaat cgctgaatat tccttttgtc tccgaccatc      120
aggcacctga gtcgctgtct ttttcgtgac attcagttcg ctgcgctcac ggctctggca      180
gtgaatgggg gtaaatggca ctacaggcgc cttttatgga ttcatgcaag gaaactaccc      240
ataatacaag aaaagcccg caccggcttc tcaggcggtt ttatggcggg tctgctatgt      300
ggtgctatct gactttttgc tgttcagcag ttcctgccct ctgattttcc agtctgacca      360
cttcggatta tcccgtaga cgtcattcag actggctaata gcaccagta aggcagcggc      420
atcatcaacg gggctcgacg ctacagtggaa cgaaaactca cgttaaggga ttttggatcat      480
gagattatca aaaaggatct tcacctagat ctttttaaat taaaaatgaa gttttaaatc      540
aatctaaagt atatatgagt aaacttggtc tgacagttaa tcttatggct cttgatctta      600
tcagtgaagc atcaagacta acaaacaaaa gtagaacaac tgttcaccgt tacatatcaa      660
agggaaaact gtccatatgc acagatgaaa acgggtgtaaa aaagatagat acatcagagc      720
ttttcagagt ttttgggtgca ttcaaagctg ttcaccatga acagatcgac aatgtaacag      780
atgaacagca tgtaaacact aatagaacag gtgaaaccag taaaacaaag caactagaac      840
atgaaattga acacctgaga caacttggtt cagctcaaca gtcacacata gacagcctga      900
aacaggcgat gctgcttata gaatacaaac tgccgacaac acgggagcca gtgacgcctc      960
ccgtggggaa aaaatcatgg caattctgga agaaatagcg ctttcagccg gcaaaccggc     1020
tgaagccgga tctgcgattc tgataacaaa ctacgaacac cagaacagcc cgtttgcggg     1080
cagcaaaaacc cgtcagctgt ctcttatata catctcgttg ttccatacaa cctccttagt     1140
acatgcaacc attatcacgc ccagaggtaa aatagtcaac acgcacgggtg ttagacattt     1200
atcccttgcg gtgatatgatt taacgtatga gcacaaaaaa gaaaccatta acacaagagc     1260
agcttgagga cgcacgtcgc cttaaagcaa tttatgaaaa aaagaaaaat gaacttggct     1320
tatcccagga atctgtcgca gacaagatgg ggatggggca gtcaggcggtt ggtgctttat     1380
ttaatggcat caatgcatta aatgcttata acgcgcgatt gcttacaaaa attctcaaag     1440
ttagcgttga agaatttagc ctttcaatcg ccagagaaat ctacgagatg tatgaagcgg     1500
ttagtatgca gccgtcaatt agaagtgagt atgagtaccc tgttttttct catgttcagg     1560
cagggatgtt ctcacctgag cttagaacct ttaccaaagg tgatgcggag agatgggtaa     1620
gcacaaccaa aaaagccagt gattctgcat tctggcttga ggttgaagggt aattccatga     1680

```

-continued

ccgcaccaac	aggctccaag	ccaagcttct	ctgacggaat	gttaattctc	gttgaccctg	1740
agcaggctgt	tgagccaggt	gattttctgca	tagccagact	tgggggtgat	gagtttacct	1800
tcaagaaact	gatcagggat	agcggtcagg	tgtttttaca	accactaaac	ccacagtacc	1860
caatgatccc	atgcaatgag	agttgttccg	ttgtggggaa	agttatcgct	agtcagtggc	1920
ctgaagagac	gtttggctga	tcggcaaggt	gttctggctg	gcgcatagct	gataacaatt	1980
gagcaagaat	cttcacgaa	ttagggaat	tttcgctccc	ctcagaacat	aacatagtaa	2040
atggattgaa	ttatggaaaa	aaaaatcact	ggatatacca	ccgttgatat	atcccaatgg	2100
catcgtaaag	aacattttga	ggcatttcag	tcagttgctc	aatgtaccta	taaccagacc	2160
gttcagctgg	atattacggc	ctttttaaag	accgtaaaga	aaaataagca	caagttttat	2220
ccggccttta	ttcacattct	tgcccgcctg	atgaatgctc	atccggaatt	ccgtatggca	2280
atgaaagacg	gtgagctggg	gatatgggat	agtgttcacc	cttgttacac	cgttttccat	2340
gagcaaaactg	aaacgttttc	atcgctctgg	agtgaatacc	acgacgattt	ccggcagttt	2400
ctacacatat	attcgcaaga	tgtggcgtgt	tacggtgaaa	acctggccta	tttcctaaa	2460
gggtttattg	agaatatgtt	tttcgtctca	gccaatccct	gggtgagttt	caccagtttt	2520
gatttaaacg	tggccaatat	ggacaacttc	ttcgcccccg	ttttaccat	gggcaaatat	2580
tatacgcaag	gcgacaaggt	gctgatgccg	ctggcgattc	aggttcatca	tgccgtttgt	2640
gatggcttcc	atgtcggcag	aatgcttaat	gaattacaac	agtactgcga	tgagtggcag	2700
ggcggggcgt	aacccggcct	cagcgcgggg	ttttctttgc	ctcacgatcg	ccccaaaaac	2760
acataaccaa	ttgtatttat	tgaaaaaata	atagatacaa	ctcactaaac	atagcaattc	2820
agatctctca	cctaccaaac	aatgcccccc	tgcaaaaaat	aaattcatat	aaaaaacata	2880
cagataacca	tctcgggtga	taaattatct	ctggcgggtg	tgacataaat	accactggcg	2940
gtgatactga	gcacatcagc	aggacgcact	gaccaccatg	aaggtgacgc	tcttaaaaat	3000
taagccctga	agaagggcag	cattcaaacg	agaaggcttt	gggtgtgtgt	atacgaaacg	3060
aagcattggc	cgtaagtgcg	attccggaaa	ggagatatct	cgagaaatac	atctgcacta	3120
cgggggtacc	tctagagaaa	tcaccttag	cgaaagctaa	ggctgatact	cttccttttt	3180
caatattatt	gaagcattta	tcagggttat	tgtctcatga	gcggatacat	atttgagtgt	3240
atttagaaaa	ataaacaaat	aggggttccg	cgcacatttc	cccgaaggt	gccacctgca	3300
tcgatttatt	atgacaactt	gacggctaca	tcattcactt	tttcttcaca	accggcacgg	3360
aactcgctcg	ggctggcccc	ggtgcatttt	ttaaataccc	gcgagaagta	gagttgatcg	3420
tcaaaaccaa	cattgcgacc	gacgggtggc	ataggcatcc	gggtggtgct	caaaagcagc	3480
ttcgctggc	tgatacgttg	gtcctcgcgc	cagcttaaga	cgctaatacc	taactgctgg	3540
cggaaaagat	gtgacagacg	cgacggcgac	aagcaaacat	gctgtgcgac	gctggcgata	3600
tcaaaattgc	tgtctgccag	gtgatcgctg	atgtactgac	aagcctcgcg	taccgatta	3660
tccatcggtg	gatggagcga	ctcgttaatc	gcttccatgc	gccgcagtaa	caattgctca	3720
agcagattta	tcgccagcag	ctccgaatag	cgccttccc	cttgcccggc	gttaatgatt	3780
tgcccaaaca	ggtcgctgaa	atcggtctgg	tgcgcttcat	ccgggcgaaa	gaaccccgta	3840
ttggcaaata	ttgacggcca	gttaagccat	tcatgccagt	aggcgcgcgg	acgaaagtaa	3900
accactgggt	gataccattc	gcgagcctcc	ggatgacgac	cgtagtgatg	aatctctcct	3960
ggcgggaaca	gcaaaatata	accgggtcgg	caaacaaatt	ctcgctccctg	atttttcacc	4020
acccctgac	cgcaatgggt	gagattgaga	atataacctt	tcattcccag	cggtcggctg	4080

-continued

ataaaaaaat	cgagataaac	gttggcctca	atcggcggtta	aacccgccac	cagatgggca	4140
ttaaaccgagt	atcccggcag	caggggatca	ttttgcgctt	cagccatact	tttcatactc	4200
ccgccattca	gagaagaaac	caattgtcca	tattgcatca	gacattgccg	tcactgcgtc	4260
ttttactggc	tcttctcgct	aaccaaaccg	gtaacccgcg	ttattaaaag	cattctgtaa	4320
caaagcggga	ccaaagccat	gacaaaaacg	cgtaacaaaa	gtgtctataa	tcacggaaga	4380
aaagtocaca	ttgattatct	gcacggcgct	acactttgct	atgccatagc	atttttatcc	4440
ataagattag	cggatcctac	ctgacgcttt	ttatcgcaac	tctctactgt	ttctccatac	4500
ccgttttttt	gggaattcga	gctctaagga	ggttataaaa	aatggatatt	aatactgaaa	4560
ctgagatcaa	gcaaaagcat	tcactaaccc	cctttcctgt	tttcctaata	agcccgcat	4620
ttcgcggggc	atattttcc	agctatttca	ggagttcagc	catgaacgct	tattacattc	4680
aggatcgtct	tgaggctcag	agctggggcg	gtcactacca	gcagctcgcc	cgtgaagaga	4740
aagaggcaga	actggcgagc	gacatggaaa	aaggcctgcc	ccagcacctg	tttgaatcgc	4800
tatgcatcga	tcattttgca	cgccacgggg	ccagcaaaaa	atccattacc	cgtgcgtttg	4860
atgacgatgt	tgagtttcag	gagcgcatgg	cagaacacat	ccggtacatg	gttgaaacca	4920
ttgctcacca	ccaggttgat	attgattcag	aggataaaaa	cgaatgagta	ctgcactcgc	4980
aacgtgggct	gggaagctgg	ctgaacgtgt	cggcatggat	tctgtcgacc	cacaggaact	5040
gatcaccact	cttcgccaga	cggcatttaa	aggatgatgc	agcgatcgcc	agttcatcgc	5100
attactgac	gttgccaacc	agtacggcct	taatccgtgg	acgaaagaaa	tttacgcctt	5160
tccctgataag	cagaatggca	tcgttccggg	ggtggggcgt	gatggctggg	cccgcatcat	5220
caatgaaaac	cagcagtttg	atggcatgga	ctttgagcag	gacaatgaat	cctgtacatg	5280
ccggaattac	cgcaaggacc	gtaatcatcc	gatctgcgtt	accgaatgga	tggaatgaatg	5340
ccgcgcgcaa	ccattcaaaa	ctcgcgaagg	cagagaaatc	acggggccgt	ggcagtcgca	5400
tcccaaacgg	atgttacgtc	ataaagccat	gattcagtgt	gcccgctctg	ccttcggatt	5460
tgctgggtatc	tatgacaagg	atgaagccga	gcgcattgtc	gaaaatactg	catacactgc	5520
agaacgtcag	ccggaacgcg	acatcactcc	ggttaacgat	gaaaccatgc	aggagattaa	5580
cactctgctg	atcgccctgg	ataaaacatg	ggatgacgac	ttattgccgc	tctgttccca	5640
gatatttctc	cgcgacattc	gtgcacgtc	agaactgaca	caggccgaag	cagtaaaagc	5700
tcttgggattc	ctgaacaga	aagccgcaga	gcagaagggtg	gcagcatgac	accggacatt	5760
atcctgcagc	gtaccgggat	cgatgtgaga	gctgtcgaa	agggggatga	tgctgggcac	5820
aaattacggc	tcggcgctcat	caccgcttca	gaagtccaca	acgtgatagc	aaaaccccg	5880
tccgaaaaga	agtggcctga	catgaaaatg	tccactctcc	acacctgct	tgtgaggtt	5940
tgcaccgggtg	tggtcccgga	agttaacgct	aaagcactgg	cctggggaaa	acagtacgag	6000
aacgacgcca	gaaccctggt	tgaattcact	tccggcgtga	atgttactga	atccccgatc	6060
atctatcgcg	acgaaagtat	gcgtaccgcc	tgctctcccg	atggtttatg	cagtgcggc	6120
aacggccttg	aactgaaatg	cccgcttacc	tcccgggatt	tcatgaagtt	ccggctcggt	6180
ggtttcgagg	ccataaagtc	agcttacatg	gcccggtgc	agtacagcat	gtgggtgacg	6240
cgaaaaaatg	cctggtaact	tgccaactat	gacccgcgta	tgaagcgtga	aggcctgcat	6300
tatgtcgtga	ttgagcggga	tgaaggtac	atggcgagtt	ttgacgagat	cgtgccggag	6360
ttcatcgaaa	aaatggacga	ggcactggct	gaaattgggt	ttgtatttgg	ggagcaatgg	6420

-continued

cgatgacgca	tcctcacgat	aatatccggg	taggcgcaat	cactttcgtc	tactccgtta	6480
caaagcgagg	ctgggtat	cccggccttt	ctgttatccg	aaatccactg	aaagcacagc	6540
ggctggctga	ggagataaat	aataaacgag	gggctgtatg	cacaaagcat	cttctgttga	6600
gttaagaacg	agtatcgaga	tggcacatag	ccttgcctca	attggaatca	ggtttgtgcc	6660
aataccagta	gaaacagacg	aagaatccat	gggtatggac	agttttccct	ttgatatgta	6720
acggtgaaca	gttggtctac	ttttgtttgt	tagtcttgat	gcttcactga	tagatacaag	6780
agccataaga	acctcagatc	cttccgtatt	tagccagtat	gttctctagt	gtggttcgtt	6840
gtttttgcgt	gagccatgag	aacgaaccat	tgagatcata	cttactttgc	atgtcactca	6900
aaaattttgc	ctcaaaactg	gtgagctgaa	tttttgcagt	taaagcatcg	tgtagtgttt	6960
ttcttagtcc	gttatgtagg	taggaatctg	atgtaatggg	tgttggtatt	ttgtcaccat	7020
tcatttttat	ctgggtgttc	tcaagtccgg	ttacgagatc	catttgtcta	tctagttaa	7080
cttgaaaaat	caacgtatca	gtcggggcgc	ctcgcttacc	aaccaccaat	ttcatattgc	7140
tgtaagtgtt	taaatcttta	cttattgggt	tcaaaaccca	ttggttaagc	cttttaaact	7200
catggtagtt	attttcaagc	attaacatga	acttaaattc	atcaaggcta	atctctatat	7260
ttgccttggt	agttttcttt	tgtgtagttt	cttttaataa	ccactcataa	atcctcatag	7320
agtatttggt	ttcaaaagac	ttaacatggt	ccagattata	ttttatgaat	ttttttaact	7380
ggaaaagata	aggcaatatc	tcttcactaa	aaactaattc	taatttttcg	cttgagaact	7440
tggcatagtt	tgtccactgg	aaaatctcaa	agcctttaac	caaaggattc	ctgatttcca	7500
cagttctcgt	catcagctct	ctggttgctt	tagctaatac	accataagca	ttttccctac	7560
tgatgttcat	catctgaacg	tattggttat	aagtgaacga	taccgtccgt	tctttccttg	7620
tagggtttcc	aatcgtgggg	ttgagtagtg	ccacacagca	taaaattagc	ttgggttcat	7680
gctccgttaa	gtcatagcga	ctaactcgta	gttcatttgc	tttgaaaaca	actaattcag	7740
acatacatct	caattggtct	aggtagtttt	aatcactata	ccaattgaga	tgggctagtc	7800
aatgataatt	actagtccct	ttcctttgag	ttgtgggtat	ctgtaaattc	tgctagacct	7860
ttgctggaaa	acttgtaaat	tctgctagac	cctctgtaaa	ttccgctaga	cctttgtgtg	7920
ttttttttgt	ttatatccaa	gtgggtataa	tttatagaat	aaagaaagaa	taaaaaaaga	7980
taaaaagaat	agatcccagc	cctgtgtata	actcactact	ttagtcagtt	ccgcagtatt	8040
acaaaaggat	gtcgcaaac					8059

<210> SEQ ID NO 88

<211> LENGTH: 2796

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: P346bb

<400> SEQUENCE: 88

atcccagaaa	agaccctgcc	gcatgcgcgt	accgacccca	atacccgatt	ccgacaccgt	60
agcactgtga	cccggccaac	tcccaccatt	tgtttcccaa	tgtgcgccac	cccatctcgg	120
gaagcttggg	cccgaacaaa	aactcatctc	agaagaggat	ctgaatagcg	ccgtcgacca	180
tcacatcatc	catcattgag	tttaaacggt	ctccagcttg	gctgttttgg	cggatgagag	240
aagattttca	gcctgatata	gattaaatca	gaacgcagaa	gcggtctgat	aaaacagaat	300
ttgcctggcg	gcagtagcgc	gggtgtccca	cctgacccca	tgccgaactc	agaagtgaag	360
cgcctagcgc	ccgatggtag	tgtgggggtc	ccccatgcga	gagtagggaa	ctgccaggca	420

-continued

tcaaataaaa	cgaaaggctc	agtcgaaaga	ctgggccttt	cgttttatct	gtgtttgtc	480
ggtgaactgg	atccttactc	gagtctagac	tgcaggett	ctcgetcact	gactcgetgc	540
gctcggtcgt	tcggctgcgg	cgagcggat	cagctcactc	aaaggcgga	atacggttat	600
ccacagaatc	aggggataac	gcaggaaaga	acatgtgagc	aaaaggccag	caaaaggcca	660
ggaaccgtaa	aaaggccg	ttgctggcgt	ttttccatag	gctccgcccc	cctgacgagc	720
atcacaaaaa	tcgacgtca	agtcagaggt	ggcgaaaccc	gacaggacta	taaagatacc	780
aggcgtttcc	ccctggaagc	tcctcgtgc	gctctcctgt	tcgaccctg	ccgcttaccg	840
gatacctgtc	cgcctttctc	ccttcgggaa	gcgtggcgct	ttctcatagc	tcacgtgtga	900
ggtatctcag	ttcgggtgag	gtcgttcgct	ccaagctggg	ctgtgtgcac	gaaccccccg	960
ttcagcccg	ccgctgcgcc	ttatccggt	actatcgtct	tgagtccaac	ccggtaagac	1020
acgacttata	gccactggca	gcagccactg	gtaacaggat	tagcagagcg	aggtatgtag	1080
gcggtgtctac	agagtctctg	aagtgggtggc	ctaactacgg	ctacactaga	aggacagtat	1140
ttggtatctg	cgtctctctg	aagccagtta	ccttcggaaa	aagagttggg	agctcttgat	1200
ccggcaaaaa	aaccaccgct	ggtagcgggt	gtttttttgt	ttgcaagcag	cagattacgc	1260
gcagaaaaaa	aggatctcaa	gaagatcctt	tgatcttttc	tacggggctc	gacgctcagt	1320
ggaacgaaaa	ctcacgttaa	gggattttgg	tcattgagatt	atcaaaaagg	atcttcacct	1380
agatcctttt	aaattaaaaa	tgaagtttta	aatcaatcta	aagtatatat	gagtaaaact	1440
ggtctgacag	ttaccaatgc	ttaatcagtg	aggcacctat	ctcagcgatc	tgtctatttc	1500
gttcatccat	agttgcctga	ctccccgtcg	tgtagataac	tacgatacgg	gagggcttac	1560
catctggccc	cagtgtctga	atgataccgc	gagacccacg	ctcaccggct	ccagatttat	1620
cagcaataaa	ccagccagcc	ggaaggggcg	agcgagaag	tggtcctgca	actttatccg	1680
cctccatcca	gtctattaat	tgttgccggg	aagctagagt	aagtagttcg	ccagttaata	1740
gtttgcgcaa	cgttggttgc	attgctacag	gcacgtggg	gtcacgctcg	tcgtttggta	1800
tggcttcatt	cagctccgg	tcccaacgat	caaggcgagt	tacatgatcc	cccatgttgt	1860
gcaaaaaagc	gggttagctc	ttcggctctc	cgatcgttgt	cagaagtaag	ttggccgag	1920
tgttatcact	catggttatg	gcagcactgc	ataattctct	tactgtcatg	ccatccgtaa	1980
gatgtctttc	tgtgactgg	gagtactcaa	ccaagtcatt	ctgagaatag	tgtatgcggc	2040
gaccgagttg	ctcttgccc	gcgtcaatac	gggataatac	cgcgccacat	agcagaactt	2100
taaaagtgtc	catcattgga	aaacgttctt	cggggcgaaa	actctcaagg	atcttaccgc	2160
tgttgagatc	cagttcgatg	taaccactc	gtgcacccaa	ctgatcttca	gcattcttta	2220
ctttcaccag	cgtttctggg	tgagcaaaaa	caggaaggca	aaatgccgca	aaaaagggaa	2280
taagggcgac	acggaaatgt	tgaatactca	tactcttcct	ttttcaatat	tattgaagca	2340
tttatcaggg	ttattgtctc	atgagcggat	acatatttga	atgtatttag	aaaaataaac	2400
aaataggggt	tccgcgcaca	tttcccgaa	aagtgccacc	tgacgtctaa	gaaaccatta	2460
ttatcatgac	attaacctat	aaaaataggc	gtatcacgag	gcagaatttc	agataaaaaa	2520
aatccttagc	tttcgctaag	gatgatttct	ggtctcacct	accaaacaat	gccccctgc	2580
aaaaaataaa	ttcatataaa	aaacatacag	ataaccatct	gcggtgataa	attatctctg	2640
gcggtgttga	cataaatacc	actggcggtg	atactgagca	catcagcagg	acgcaactgac	2700
caccatgaag	gtgacgtctc	taaaaattaa	gccctgaaga	agggcagcat	tcaaagcaga	2760

-continued

aggttttggg gtgtgtgata cgaacgaag cattgg 2796

<210> SEQ ID NO 89
 <211> LENGTH: 9232
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MAD70 EXPRESSION VECTOR

<400> SEQUENCE: 89

gcgatcgagg ctcccgacat cttggaccat tagctccaca ggtatcttct tccctctagt 60
 ggtcataaca gcagcttcag ctacctctca attcaaaaaa cccctcaaga cccgtttaga 120
 ggccccaagg ggttatgcta tcaatcgttg cgttacacac acaaaaaacc aacacacatc 180
 catcttcgat gtagtagcat tttattatct aactgctgat cgagtgtagc cagatctata 240
 gtaatcaatt acggggatcat tagttcatag cccatataatg gagttccgag ttacataact 300
 tacggttaaa ggcccgctg gctgacggcc caacgacccc cgccattga cgtcaataat 360
 gacgtatgtt cccatagtaa cgccaatagg gactttccat tgacgtcaat ggggtgacta 420
 tttacggtaa actgcccact tggcagtaca tcaagtgtat catatgcca gtacgcccc 480
 tattgacgtc aatgacggta aatggccgag ctggcattat gccagtaga tgacctatg 540
 ggactttcct acttggcagt acatctacgt attagtcac gctattacca tgcgtcgagg 600
 tgagcccccac gttctgcttc actctcccca tctccccccc ctccccccc ccaattttgt 660
 atttatttat tttttaatta ttttatgcag cgatgggggg gggggggggg gggggcgagc 720
 ccaggcgagg cgggcgagg cgaggggagg ggcggggcga ggcggagagg tgcggcgga 780
 gccaatcaga gcggcgagct ccgaaagttt ccttttatgg cgaggcgagg gcggcgagg 840
 cccataaaaa agcgaagcgc gcggcgagg gtagtcgctg cgttgcttc gcccggtgac 900
 ccgctccgag ccgctccgag ccgcccggcc cggctctgac tgaccgctt actccacag 960
 gtgagcgggg gggacggccc ttctccctcc gggctgtaat tagcgttggt tttaatgacg 1020
 gctcgtttct tttctgtggc tgcgtgaaag ccttaaggag ctccgggagg gcctttgtgc 1080
 gggggggagg ggctcgagg gtgcgtgctg gtgtgtgtgc gtggggaggc ccgctgagc 1140
 ccgctgctgc ccggcgagtg tagcgtctgc gggcgagcgc cggggctttg tgcgtccgc 1200
 gtgtgcgcga ggggagcgc ggccgggggc ggtgccccgc ggtgcggggg ggtgcgagg 1260
 ggaacaaagg ctgcgtgagg ggtgtgtgct tgggggggtg agcagggggt gtggcgagg 1320
 cggctcgggt gtaaccccc cctggcacc cctccccga gttgctgagc acggcccggc 1380
 ttccgggtgag gggctccgtg cggggcggtg ccgggggctc gccgtgccc ggggggggtg 1440
 gcggcaggtg ggggtgccc gcggggcggg gccgcctcgc gccgggagg gctcggggga 1500
 gggcgagcgc gggcccgagg cgcggcgagg tgtcagaggc cggcgagcgc cagccattgc 1560
 cttttatggt aatcgtgcga gaggcgagc ggaattcctt tgtcccaaat ctggcgagg 1620
 cgaaatctgg gaggcgcgc cgcacccctc ctacgggggc cgggcgaagc ggtgcggcgc 1680
 cggcaggaa gaaatgggc gggagggcct tcgtgcgtgc ccgcgcgcgc gtccctctct 1740
 ccatctccag cctcggggct gccgcagggg gacggctgcc ttccgggggg acggggcagg 1800
 gcgggggttc gcttctgggc tgtgacgggc ggcttttagg cctctgctaa ccatgttcat 1860
 gccttctct ttttcttaca gctcctgggc aacgtgctgg ttgtgtgct gtctcatcat 1920
 tttggcaaag atctttgtgc atcctaccat ccaactgcga caccgccag cggccgctgc 1980
 caagcttcgc agctctcgaa ttcaaaggag gtaccacat gaacaacgc acaataatt 2040

-continued

ttcagaactt catcgggatc tcaagtttgc agaaaacgct gcgcaatgct ctgatcccca	2100
cggaaaccac gcaacagttc atcgtaaga acggaataat taaagaagat gagttacgtg	2160
gcgagaaccg ccagattctg aaagatatca tggatgacta ctaccgcgga ttcattctctg	2220
agactctgag ttctattgat gacatagatt ggactagcct gttcgaaaa atggaaattc	2280
agctgaaaaa tgggtataat aaagatacct taattaagga acagacagag tatcgaaaag	2340
caatccataa aaaatttgcg aacgacgac gggttaagaa catgtttagc gccaaactga	2400
ttagtgcacat attacctgaa ttgtcatcc acaacaataa ttattcggca tcagagaaa	2460
aggaaaaaac ccaggtgata aaattgtttt cgcgctttgc gactagcttt aaagattact	2520
tcaagaaccg tgcaaatgac ttttcagcgg acgatatttc atcaagcagc tgccatcgca	2580
tcgtcaacga caatgcagag atattctttt caaatgcgct ggtctaccgc cggatcgtaa	2640
aatcgctgag caatgacgat atcaacaaaa ttccgggcga tatgaaagat tcattaaaag	2700
aatgagtcct ggaagaaata tattcttacg agaagtatgg ggaatttatt acccaggaag	2760
gcattagctt ctataatgat atctgtggga aagtgaattc ttttatgaac ctgtattgtc	2820
agaaaaataa agaaaacaaa aatttataca aacttcagaa acttcacaaa cagattctat	2880
gcattgcgga cactagctat gaggtcccgt ataaatttga aagtgcgag gaagtgtacc	2940
aatcagttaa cggcttcctt gataacatta gcagcaaaaa tatagtcgaa agattacgca	3000
aaatcggcga taactataac ggctacaacc tggataaaat ttatatcgtg tccaaatttt	3060
acgagagcgt tagccaaaaa acctaccgcg actgggaaac aattaatacc gccctcgaaa	3120
ttcattacaa taatatcttg ccgggtaacg gtaaaagtaa agccgacaaa gtaaaaaaag	3180
cggttaagaa tgatttacag aaatccatca ccgaaataaa tgaactagtg tcaaactata	3240
agctgtgcag tgacgacaac atcaaacggg agacttatat acatgagatt agccatatct	3300
tgaataactt tgaagcacag gaattgaaat acaatccgga aattcaccta gttgaatccg	3360
agctcaaacg gagtgagctt aaaaacgtgc tggacgtgat catgaatgcg tttoattggt	3420
gttcggtttt tatgactgag gaacttgttg ataaagacaa caatttttat gcggaactgg	3480
aggagattta cgtatgaaat tatccagtaa ttagtctgta caacctgggt cgtaactacg	3540
ttaccacaga accgtacagc acgaaaaaga ttaaatgaa ctttgaata ccgacgttag	3600
cagacggttg gtcaaatgcc aaagagtatt ctaataacgc tatcatactg atgcgcgaca	3660
atctgtatta tctgggcacg tttaatgcga agaataaacg ggacaagaag attatcgagg	3720
gtaatacgtc agaaaataag ggtgactaca aaaagatgat ttataatttg ctcccgggtc	3780
ccaacaaaat gatcccgaaa gttttcttga gcagcaagac ggggggtggaa acgtataaac	3840
cgagcgccta tatctagag gggataaac agaataaaca tatcaagtct tcaaaagact	3900
ttgatatac tttctgcat gatctgatcg actacttcaa aaactgtatt gcaattcatc	3960
ccgagtggaa aaacttcggt ttgatattta gcgacaccag tacttatgaa gacatttccg	4020
ggttttatcg tgaggtagag ttacaagggt acaagattga ttggacatac attagcgaaa	4080
aagacattga tctgctgcag gaaaaaggtc aactgtatct gttccagata tataacaaaag	4140
atttttcgaa aaaatcaacc gggaatgaca accttcacac catgtacctg aaaaatcttt	4200
tctcagaaga aaatcttaag gatatcgacc tgaaccttaa cggcgaagcg gaaatcttct	4260
tcaggaagag cagcataaag aacccaatca ttcataaaaa aggctcgatt ttagtcaacc	4320
gtacctacga agcagaagaa aaagaccagt ttggcaacat tcaaatgtg cgtaaaaaata	4380

-continued

ttccggaaaa	catttatcag	gagctgtaca	aatacttcaa	cgataaaaagc	gacaaaagagc	4440
tgtctgatga	agcagccaaa	ctgaagaatg	tagtgggaca	ccacgaggca	gcgacgaata	4500
tagtcaagga	ctatcgctac	acgtatgata	aatacttcct	tcatatgcct	attacgatca	4560
atttcaaagc	caataaaaacg	ggttttatta	atgataggat	cttacagtat	atcgctaaag	4620
aaaaagactt	acatgtgatc	ggcattgatc	ggggcgagcg	taacctgatc	tacgtgtccg	4680
tgattgatac	ttgtggaat	atagtgaac	agaaaagctt	taacattgta	aacggctacg	4740
actatcagat	aaaactgaaa	caacaggagg	gcgctagaca	gattgcgcg	aaagaatgga	4800
aagaaattgg	taaaattaaa	gagatcaaag	agggtacct	gagcttagta	atccacgaga	4860
tctctaaaat	ggtaatcaaa	tacaatgcaa	ttatagcgat	ggaggatttg	tcttatgggt	4920
ttaaaaaagg	gcgctttaag	gtcgaacggc	aagtttacca	gaaatttgaa	accatgctca	4980
tcaataaact	caactatctg	gtattttaaag	atatttcgat	taccgagaat	ggcggctctc	5040
tgaaagggtta	tcagctgaca	tacattcctg	ataaacttaa	aaacgtgggt	catcagtgcg	5100
gctgcatttt	ttatgtgcct	gctgcataca	cgagcaaaaat	tgatccgacc	accggctttg	5160
tgaatatctt	taaattttaa	gacctgacag	tgacgcaaaa	acgtgaattc	attaaaaaat	5220
ttgactcaat	tcgttatgac	agtgaaaaaa	atctgttctg	ctttacattt	gactacaata	5280
actttattac	gcaaaacacg	gtcatgagca	aatcatcgtg	gagtgtgtat	acatacggcg	5340
tgcgcatcaa	acgtcgcttt	gtgaacggcc	gcttctcaaa	cgaaagtgat	accattgaca	5400
taaccaaaga	tatggagaaa	acgttggaaa	tgacggacat	taactggcgc	gatggccacg	5460
atcttcgtoa	agacattata	gattatgaaa	ttgttcagca	catattcgaa	attttccggt	5520
taacagtga	aatgcgtaac	tccttgtctg	aactggagga	ccgtgattac	gatcgtctca	5580
tttcacctgt	actgaacgaa	aataacattt	tttatgacag	cgcgaaagcg	ggggatgcac	5640
ttcctaagga	tgccgatgca	aatggtgcgt	attgtattgc	attaaaagg	ttatatgaaa	5700
ttaaacaaat	taccgaaaat	tggaagaag	atggtaaatt	ttcgccgat	aaactcaaaa	5760
tcagcaataa	agattggttc	gactttatcc	agaataagcg	ctatctcctc	tacctcgagg	5820
tttcgaagag	accggcggcc	acaaaaaag	ctgggcaggc	aaaaagaag	aagggttctg	5880
ggaagccgat	acctaacca	ctcctcgggc	ttgacagcac	ataactagaa	gttgtctcct	5940
cctgcactga	ctgactgata	caatcgattt	ctggatccgc	aggcctctgc	tagcttgact	6000
gactgagata	cagcgtaacct	tcagctcaca	gacatgataa	gatacattga	tgagtttgga	6060
caaaccacaa	ctagaatgca	gtgaaaaaaa	tgctttattt	gtgaaatttg	tgatgctatt	6120
gctttatttg	taaccattat	aagctgcaat	aaacaagtta	acaacaacaa	ttgcattcat	6180
tttatgtttc	aggttcagg	ggaggtgtgg	gaggtttttt	aaagcaagta	aaacctctac	6240
aatgtggta	ttggcccatc	tctatcggt	tcgtagcata	accccttggg	gcctctaaac	6300
gggtcttgag	gggttttttg	tgccctcgg	gccggattgc	tatctaccgg	cattggcgca	6360
gaaaaaatg	cctgatgoga	cgctgcgcgt	cttatactcc	cacatagcc	agattcagca	6420
acggatacgg	cttcccaaac	ttgccactt	ccatacgtgt	cctccttacc	agaaatttat	6480
ccttaaggtc	gtcagctatc	ctgcaggcga	tctctcgatt	tcgatcaaga	cattccttta	6540
atggtctttt	ctggacacca	ctaggggtca	gaagtagttc	atcaaaacttt	cttccctccc	6600
taatctcatt	ggttaccttg	ggctatcgaa	acttaattaa	ccagtcaagt	cagctacttg	6660
gcgagatcga	cttgtctggg	tttcgactac	gctcagaatt	gcgtcagtc	agttcgatct	6720
ggtccttgct	attgcaccg	ttctccgatt	acgagtttca	tttaaatcat	gtgagcaaaa	6780

-continued

ggccagcaaa aggccaggaa ccgtaaaaag gccgcgttgc tggcggtttt ccataggctc	6840
cgccccctg acgagcatca caaaaatcga cgctcaagtc agaggtggcg aaacccgaca	6900
ggactataaa gataccaggc gtttccccct ggaagctccc tcgtgcgctc tcctgttccg	6960
accctgccgc ttaccggata cctgtccgcc tttctccctt cggaagcggt ggcgctttct	7020
catagctcac gctgtaggta tctcagttcg gtgtaggctg ttcgctccaa gctgggctgt	7080
gtgcacgaac ccccggttca gcccgaccgc tgcgccttat ccgtaacta tcgtcttgag	7140
tccaacccgg taagacacga cttatcgcca ctggcagcag ccactggtaa caggattagc	7200
agagcgaggt atgtaggcgg tgctacagag ttcttgaagt ggtggcctaa ctacggctac	7260
actagaagaa cagtatttgg tatctgcgct ctgctgaagc cagttacctt cggaaaaaga	7320
gttggtagct cttgatccgg caaacaacc accgctggta gcggtggtt tttgtttgc	7380
aagcagcaga ttacgcgcag aaaaaagga tctcaagaag atcctttgat cttttctacg	7440
gggtctgacg ctcagtggaa cgaaaactca cgtaaggga ttttggtcat gagattatca	7500
aaaaggatct tcacctagat ctttttaaat taaaaatgaa gttttaaatc aatctaaagt	7560
atatatgagt aaacttggtc tgacagttac caatgcttaa tcagtgggc acctatctca	7620
gcgatctgtc tatttcgttc atccatagtt gcatttaaat ttccgaactc tccaaggccc	7680
tcgtcggaaa atcttcaaac ctttcgtccg atccatcttg caggctacct ctgaaacgaa	7740
ctatcgcaag tctcttggtc ggcttgccgc cttggtatt gcttggcagc gcctatcgcc	7800
aggtattact ccaatccoga atatccgaga tcgggatcac ccgagagaag ttcaacctac	7860
atcctcaatc ccgatctatc cgagatccga ggaatatcga aatcggggcg cgctgggtgt	7920
accgagaacg atcctctcag tgcgagtctc gacgatccat atcgttgctt ggcagtcagc	7980
cagtcggaat ccagcttggg acccaggaag tccaatcgtc agatattgta ctcaagcctg	8040
gtcacggcag cgtaccgacg tgtttaaac tagatattga tagtctgac ggtcaacgta	8100
taatcgagtc ctagcttttg caaacatcta tcaagagaca ggatcagcag gaggtttcg	8160
catgattgaa caagatggat tgcaacgagg ttctccggcg gcttgggtgg agaggctatt	8220
cggtatgac tgggcacaac agacaatcgg ctgctctgat gccgcgtgt tcgggtgtc	8280
agcgcagggg cgtccggttc tttttgtcaa gaccgacctg tccggtgcc tgaatgaact	8340
gcaagacgag gcagcgcggc tatcgtggct ggcgacgac ggcgttcctt gcgcggctgt	8400
gctcgacgtt gtcactgaag cgggaaggga ctggctgcta ttgggcgaag tgccggggca	8460
ggatctcctg tcctctcacc ttgctcctgc cgagaaagta tccatcatgg ctgatgcaat	8520
gcggcggctg catacgcttg atccggctac ctgcccattc gaccaccaag cgaaacatcg	8580
catcgagcga gcacgtactc ggatggaagc cggctttgtc gatcaggatg atctggacga	8640
agagcatcag gggctcgcgc cagccgaact gtccgccagg ctcaaggcgt ctatgccga	8700
cggcgaggat ctgctctgta cccacggcga tgcctgcttg ccgaatatca tggtgaaaaa	8760
tggcgcgttt tctggattca tcgactgtgg ccgtctgggt gtggcgacc gctatcagga	8820
catagcgttg gctaccctg atattgctga agagcttggc ggcaaatggg ctgaccgctt	8880
ccttggtctt tacggtatcg ccgcgccga ttcgcagcgc atcgcttct atcgcttct	8940
tgacgagttc ttctgaccga ttctaggtgc attggcgag aaaaaatgc ctgatgcgac	9000
gtgcgcgtc ttatactccc acatatgcca gattcagcaa cggatacggc ttcccaact	9060
tgcccacttc catacgtgtc ctccctacca gaaatttata cttaaggctg tttaaactcg	9120

-continued

actctggctc tatcgaatct ccgtcgtttc gagcttacgc gaacagccgt ggcgctcatt	9180
tgctcgtcgg gcacgaatc tcgtcagcta tcgtcagctt acctttttgg ca	9232

<210> SEQ ID NO 90
 <211> LENGTH: 3064
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: GRNA EXPRESSION VECTOR FOR MAMMALIAN CELLS

<400> SEQUENCE: 90

tcgcgcgttt cgggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca	60
cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg	120
ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc	180
accaaatacg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc	240
attcgccatt caggctgcgc aactgttggg aaggcgcatc ggtgcgggccc tcacgctat	300
tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt	360
tttcccgatc acgacgttgt aaaacgacgg ccagtgcac gcgatgacga tggatagcga	420
ttcatcgatg agctgacccg atcgccgcgc cggagggtt gcgtttgaga cgggcgacag	480
atgagggcct atttcccatg attccttcat atttgcata acgatacaag gctgttagag	540
agataaattg aattaatttg actgtaaaaca caaagatatt agtacaaaat acgtgacgta	600
gaaagtaata atttcttggg tagtttgacg ttttaaaatt atgttttaaa atggactatc	660
atatgcttac cgtaacttga aagtatttcg atttcttggc tttatatatc ttgtggaaag	720
gacgaaacac cgtcaaaaga cctttggaat ttctactctt gtagatccgt caccaaaatc	780
agattcattt ttttatcagt tctggaccag cgagctgtgc tgcgactcgt ggcgtaata	840
tggtcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga	900
gccggaagca taaagtgtaa agcctggggt gcctaagtgc tgagctaact cacattaatt	960
gcgttgccgt cactgcccgc tttccagtcg ggaacctgt cgtgccagct gcattaatga	1020
atcgcccaac gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttcctcgctc	1080
actgactcgc tgcgctcggc cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg	1140
gtaatacggc tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc	1200
cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc	1260
ccccctgacg agcatcaca aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga	1320
ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc	1380
ctgtcgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat	1440
agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg	1500
cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc	1560
aaccgcgtta gacacgaatt atcgccactg gcagcagcca ctggtaacag gattagcaga	1620
gcgaggtatg taggcggtgc tacagagttc ttgaagtggg ggcctaacta cggtacact	1680
agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt	1740
ggtagctctt gatccggcaa acaaacacc cgtggtagcg gtggtttttt tgtttgcaag	1800
cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg	1860
tctgacgctc agtggaacga aaactcacgt taagggattt tggatcatgag attatcaaaa	1920
aggatcttca cctagatcct tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata	1980

-continued

tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg	2040
atctgtctat ttcgttcac ccatagttgcc tgactccccg tcgtgtagat aactacgata	2100
cgggagggct taccatctgg cccacgtgct gcaatgatac cgcgagaccc acgctcaccg	2160
gtccagatt tatcagcaat aaaccagcca gccggaagg cgcagcgcag aagtggctct	2220
gcaactttat ccgcctccat ccagctctatt aattgttgcc gggaagctag agtaagtagt	2280
tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc	2340
tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga	2400
tcccccatgt tgtgcaaaaa agcgggttagc tccttcgggc ctccgatcgt tgtcagaagt	2460
aagttggcgc cagtgttatc actcatgggt atggcagcac tgcataattc tcttactgtc	2520
atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa	2580
tagtgatatgc ggcgaccgag ttgctcttgc ccggcgtaaa tacgggataa taccgcgcca	2640
catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt ctccggggcg aaaactctca	2700
aggatcttac cgctgttgag atccagttcg atgtaaccca ctctgcacc caactgatct	2760
tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc	2820
gcaaaaaagg gaataagggc gacacggaat tggtgaatac tcatactcta cttttttcaa	2880
tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt	2940
tagaaaaata aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc	3000
taagaaacca ttattatcat gacattaacc tataaaaaata ggcgatatcac gagggccttt	3060
cgtc	3064

We claim:

1. An engineered nucleic acid-guided nuclease with a lowered cutting activity relative to the nucleic acid-guided nuclease having the sequence of SEQ ID No. 1, wherein the engineered nucleic acid-guided nuclease has a sequence comprising any of SEQ ID Nos. 8, 9, 10 or 15.

2. The engineered nucleic acid-guided nuclease of claim 1 comprising SEQ ID No. 8.

3. The engineered nucleic acid-guided nuclease of claim 1 comprising SEQ ID No. 9.

4. The engineered nucleic acid-guided nuclease of claim 1 comprising SEQ ID No. 10.

5. The engineered nucleic acid-guided nuclease of claim 1 comprising SEQ ID No. 15.

6. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1.

7. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 2.

8. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 3.

35

9. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 11.

10. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 12.

40 11. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 13.

12. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 14.

45 13. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 67.

14. An enzyme cocktail comprising an engineered nucleic acid-guided nuclease of claim 1 and SEQ ID No. 68.

15. An enzyme cocktail comprising at least two of the engineered nucleic acid-guided nucleases of claim 1.

50 16. An enzyme cocktail comprising at least three of the engineered nucleic acid-guided nucleases of claim 1.

17. An enzyme cocktail comprising all of the engineered nucleic acid-guided nucleases of claim 1.

* * * * *